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ON THE RELATIONSHIP OF THE NUCLEAR MEMBRANE TO THE PROTOPLAST.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XLVII.

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(WITH PLATE XV)

THE nucleus has long been regarded as not only the most important, but with its chromatin, linin, nucleolus, karyolymph, and membrane, it is also regarded as the most complex organ of the cell. Although the literature on plant cytology is rapidly accumulating and revealing much that is of interest on the centrosome question, spindle formation, chromosome reduction, etc., we as yet know little or nothing as to the origin and development of the nucleus. Its morphology is hidden by its complexity. This apparent complexity of the nucleus is in a large measure due to the presence of the nuclear membrane. A nucleus without a membrane, as we see it in certain stages of mitosis, becomes a comparatively simple structure. Such a condition of the nucleus may be observed immediately preceding spindle formation in the spore mother cells of any of the higher plants. At this stage the nuclear wall disappears, the karyolymph becomes diffused throughout the cytoplasm, the nucleolus and the linin lose their identity, and the only element left which can be regarded as nuclear is the mass of chromatin. It thus becomes evident that the formation of a membrane is the most important factor which gives the nucleus its complex structure.

So far as the writer is aware, no observations have been recorded on the origin and formation of the nuclear membrane in plants. It is the object of the present work, therefore, to account for the presence of this membrane and its morphological relationship to the other nuclear elements, with the hope that it might lead to at least a partial understanding of the morphology of the nucleus itself.

In undertaking this work it was at first thought that a study of the most primitive types of plant life—where the cell is much simpler and the nuclear structures less highly differentiated than in the higher types—would be the most natural way of approaching the subject. Consequently the cell contents of a large number of forms of the Cyanophyceae and of the simpler Chlorophyceae were examined. A careful study of these forms, however, failed to give satisfactory results so far as the formation of the nuclear membrane is concerned. In the cell of the Cyanophyceae bodies were found which resembled chromatin, but in none of the forms examined could any of the other nuclear structures be identified. These chromatin-like bodies were surrounded by neither nuclear sap nor membrane. In the simpler Chlorophyceae examined the chromatin was found to be surrounded by both nuclear sap and membrane, but the nucleus was found to be much too small to follow out accurately the stages in the formation of the nuclear membrane. It has been thought advisable, therefore, to use the highly organized nucleus of the flowering plants as an object for this study. The following conclusions, therefore, are drawn mainly from observations made in the individual history of the typical nucleus of the higher plants.

MATERIAL AND METHODS.

In order to follow the sequence of events which lead to the formation of the nuclear membrane, it is desirable to obtain material with clear large nuclei, where the various stages in the development of the daughter nuclei may be distinctly observed. For this purpose the sporogenous cells of the higher plants seemed to furnish all the desired conditions.

Although the spore mother cells of *Passiflora coerulea* and the

archesporial cells of *Equisetum limosum* were especially studied, and were selected as types to illustrate the process of nuclear membrane-formation, the results obtained from these forms were confirmed by observations made on several other forms, such as Lilium, Cobaea, Gladiolus, Hedera, Pinus, Pteris, etc.

Flemming's strong solution of chromic-osmic-acetic acid, diluted with one volume of water, was used for fixing, and the triple stain, safranin, gentian-violet, and orange G, for staining. After being fixed the material was washed in running water and was then dehydrated by being passed through various grades of alcohol. Bergamot oil preceded the infiltration of paraffin. Microtome sections from 1 to 3.6 μ thick were used.

THE FORMATION OF VACUOLES AND THEIR RESEMBLANCE TO NUCLEI.

To any one who has made a special study of the nucleus, especially of the spore mother cells of the higher plants, the resemblance of this body to a vacuole has probably suggested itself. As is well known, the presence of a vacuole is one of the most striking characteristics of the plant cell. This is particularly so of the mature cells in any vegetative tissue, where the vacuole is quite as constant as the nucleus. The vacuole is not apparent in the very young cell, as the nucleus and cytoplasm seem to occupy the entire cell cavity. At an early stage in the growth of the cell, however, small lacunae containing a watery fluid may be observed in the cytoplasm. As the cell continues to grow these lacunae become much larger and finally flow together, forming one or more large vacuoles in the cell. As these vacuoles enlarge the cell continues its growth. The amount of cytoplasm, however, does not increase at the same rate as the contents of the vacuole. The result is that in the mature cell the vacuole may occupy the greater part of the cell cavity. The watery fluid of the vacuole holds many substances in solution. It may function as a storehouse for reserve food material. It may even contain solid bodies such as starch grains, aleurone grains, and crystals of calcium carbonate or oxalate. Finally the vacuole is always surrounded by a distinct limiting plasmatic membrane.

In all of these particulars the structure of the mature nucleus bears a very striking resemblance to the vacuole. It is a cavity containing a watery fluid—the nuclear sap or karyolymph—in which lie imbedded the chromatin thread and nucleoli. Structurally the nuclear sap may be compared to the cell sap of the vacuole; the chromatin and nucleoli may be compared to the solid bodies of reserve food materials found in the cell sap; and finally the nuclear membrane may be compared to the tonoplast. These two latter structures not only bear a general resemblance to each other, but, as we shall demonstrate later, their origin and method of formation are identical.

This resemblance of the nucleus to the vacuole is particularly evident in the cells of sporogenous tissue, where the vacuole is not usually present and where the nuclear cavity occupies the greater part of the cell. In many cases the nucleus was represented as constituting fully two-thirds of the cell contents (*fig. 13*), and the cytoplasm in such cases was a mere zone between the nuclear membrane and the cell wall. As we know so little in regard to the direct function of the nucleus in relation to metabolism, we are unable to account for the large size of the nuclear cavity in these sporogenous cells. But as these cells are characterized by the absence of a vacuole, this fact suggests that the nuclear cavity may here function as the vacuole functions in the vegetative cells. The writer regards this suggestion worthy of consideration, since the function of the nucleus is coming to be regarded more and more as an essential factor in constructive metabolism.

THE PRESENCE AND FORMATION OF PLASMATIC MEMBRANES IN THE CELL.

Of late years much attention has been given to the importance of plasmatic membranes and their relationship to osmosis. As a result of the investigations of Nägeli, Traube, and more particularly of Pfeffer, the cell is now regarded as an osmotic system composed of a series of membranes. In the typical plant cell there are three of these membranes commonly recognized, namely, the cell wall, the ectoplast, and the tonoplast.

Structurally the cell wall is entirely different from the ectoplast and tonoplast. Composed mainly of cellulose, it protects the inner protoplast, gives rigidity to the whole cell, and allows of a considerable internal osmotic pressure. As its method of formation has nothing in common with that of the inner plasmatic membranes, its presence will not form a part of the present discussion.

The two inner plasmatic membranes—which in the ordinary vegetative cell constitute the outer and inner limiting layers respectively of the primordial utricle—have the same general structure, and their methods of formation are identical, so far as we know. The ectoplast is present in every cell, no matter whether the cell is surrounded by a cell wall or not. It is very conspicuous in naked cells. In the ordinary cell, when not in a state of plasmolysis, the ectoplast is always found in close contact with the cell wall. As far as can be revealed by the microscope, it is merely a differentiated film of the cytoplasm.

The tonoplast is always to be found surrounding the watery fluid of the vacuole. As young cells and most sporogenous cells do not possess a vacuole, the tonoplast is not present. It is not as constant a cell structure, therefore, as the ectoplast. It is, like the ectoplast, merely a differentiated portion of the cytoplasm. Its formation may be followed readily by observing the gradual development of the vacuole which is associated with the growth of all vegetative cells. A complete series showing the gradual formation of the vacuole may be observed in any growing region, such as root tips or growing points. Accompanying the gradual accumulation of the watery contents of the vacuole, the gradual differentiation of the cytoplasm coming in contact with the cell sap becomes quite evident. Just what changes in the minute structure of the cytoplasm take place in formation of the membrane cannot be revealed by the microscope. In this connection, however, Pfeffer says as follows:

> Every mass of watery fluid present in the protoplasm must be surrounded by a vacuolar membrane to form a larger or smaller vacuole, while masses of plasma which have escaped from the cell also become clothed by a plasmatic membrane, and form large vacuolar bubbles in water, but not in plasmolysing solutions. From what has been said above, it follows that a plas-

matic membrane must be immediately formed on a freshly exposed surface as it comes into contact, not with plasma, but with other media and especially water. Nevertheless, the determining causes cannot at present be precisely defined, and it is hardly probable that the plasmatic membrane is simply the expression of the physical surface tension, which is necessarily always present. The latter may be of decisive importance, however, in the formation by means of the molecular forces exerted by it, while at the same time contact with the medium may cause the substances in the peripheral film to be precipitated in an insoluble form, which redissolves when returned to the interior of the plasma. Since a membrane is formed on every isolated fragment of plasma, even when its vital activity is reduced to its lowest ebb, it seems as if the actual exposure of a peripheral film induces the formation of the plasmatic membrane.

It is not my purpose to enter into a theoretical discussion as to the minute changes which take place during the formation of the plasmatic membranes, but merely to point out the fact that whenever the cytoplasm is exposed to a watery surface a membrane is formed and that all membranes thus formed are structurally identical, so far as can be revealed by the microscope. A third internal plasmatic membrane which has never been considered in this connection is the nuclear membrane. It too comes in contact with a watery fluid, the nuclear sap or karyolymph. As we shall see from the following observations, it is formed in identically the same manner as the tonoplast. It is nothing more than a modified film of cytoplasm, and on account of its similarity in structure and method of formation to that of the ectoplast and tonoplast the writer regards it as one of the series of plasmatic membranes of the cell.

THE FORMATION OF THE NUCLEAR MEMBRANE.

In the history of every nucleus of the higher plants which divides mitotically there are two stages in which this organ consists of nothing but chromatin. The first of these stages may be seen in any mother nucleus in its preparation for mitosis during the period of spindle formation. At this time the nuclear wall breaks down, the nucleoli and linin disappear, the karyolymph becomes diffused throughout the cytoplasm, and by the time the spindle is formed the only nuclear element that remains is the chromatin which has assumed the form of definite

chromosomes. In his works on Cobaea and Gladiolus (1898, 1900) the writer called attention to the breaking down of the nuclear membrane. Beyond stating that the observations made on these forms have been confirmed by a study of the material used in the present investigation, it will be unnecessary to describe the process farther. It might be well, however, to call attention to the fact that it seems to be a general occurrence in the higher plants that the nuclear membrane breaks down either immediately preceding or during the process of spindle formation, and that therefore the mother nucleus is at this period devoid of a membrane.

The second stage in which the nucleus is devoid of a membrane may be observed in the young daughter nuclei previous to the accumulation of the karyolymph. It is this second stage which affords us an opportunity of following the course of events which leads to the formation of a membrane around each daughter nucleus. *Fig. 1* illustrates a stage in the pollen mother cell of *Passiflora coerulea* immediately after the chromosomes have reached the poles of the spindle. Here the chromosomes are shown still attached to the connective fibers of the spindle, but otherwise lie freely in the cytoplasm. Their individual identity may be easily observed. This stage is of very short duration, however, for the chromosomes soon fuse together, forming a single mass of chromatin. This fusion of the chromosomes is shown in *fig. 2*. This large irregularly shaped mass of chromatin comes in contact with the cytoplasm at every point of its outer surface. With the hope of detecting the very first indication of the formation of the nuclear membrane, many preparations showing this stage were carefully studied, but in no case could any trace of a membrane be found during this period. The nucleus at this time consists of nothing but a large single irregularly shaped mass of chromatin.

This stage, however, like the first, is of very short duration. The chromatin undergoes a change which is accompanied by the appearance of small but very distinct lacunae within the chromatin mass. *Fig. 3* shows two of these lacunae centrally situated. This is the first indication of the accumulation of karyo-

lymph. These lacunae increase in size, flow together, and appear to force the surrounding chromatin outward. This gradual increase in the amount of karyolymph is well shown in *figs. 4* and *5*. *Fig. 4* shows a large central cavity in the chromatin mass, and the whole takes on a ring-like appearance in section. *Fig. 5* shows this still further developed, with two cavities filled with karyolymph. The chromatin here is doubtless preparing to take on the spirem condition of the ordinary resting nucleus. These stages (see *figs. 3, 4, 5, 6*) clearly show that this change in the shape of the chromatin is closely associated with the accumulation of the karyolymph. Up to the stage shown in *fig. 5* no change whatever could be detected in the cytoplasm that would indicate the formation of a membrane, although up to this time the chromatin has been in close touch, on all sides, with the cytoplasm. In *fig. 6*, however, is shown a stage in which the karyolymph has increased to such an extent and the chromatin has become so divided that the latter is no longer immediately surrounded by cytoplasm. Here for the first time the karyolymph comes in direct contact with the cytoplasm, and here also we have the first indication of a membrane. As shown in *fig. 6*, the membrane does not completely surround the chromatin, but is only observable at that region where the cytoplasm is directly exposed to the karyolymph. By the still greater increase in the amount of karyolymph which is accompanied by a still greater change in the shape of the chromatin (*figs. 7, 8*), this exposed region increases until the chromatin is completely surrounded by the karyolymph (*fig. 7*), and the latter becomes exposed to the cytoplasm on all sides. A complete series of stages was carefully examined, and it was found without exception that just as soon as a cytoplasm becomes exposed to the karyolymph there a membrane is formed, and where this exposed surface grows by the greater accumulation of the karyolymph the membrane increases accordingly. The result of this is a large central vacuolar structure (*figs. 8, 13*) containing chromatin in the spirem stage and commonly recognized as the resting condition of the mature nucleus.

While the formation of the nuclear membrane was most

carefully followed in the pollen mother cells of Passiflora, and is here taken as the type, many other forms were also studied. Among these were the pollen mother cells of Cobaea, Gladiolus, Hedera, Lilium, Smilacina, and the archesporial cells of Equisetum. In all of these forms the process of nuclear membrane-formation was found to be practically the same as that described for Passiflora. In certain cases, however, it was found that in the accumulation of the karyolymph and the dividing of the chromatin mass the process differs slightly. This was particularly evident in the archesporial cells of *Equisetum limosum*. Here, as in Passiflora, the chromosomes unite to form a single mass of chromatin at each pole of the spindle (*fig. 9*). In the next stage, however, instead of only two or three lacunae appearing in the chromatin, a large number make their appearance (*fig. 10*). These increase in number, grow independently, and do not flow together in this early stage as they do in Passiflora. The result is—as shown in *figs. 10, 11, 12*—that in section view we do not have that ring-like appearance of the chromatin which was found to be so characteristic of Passiflora.

Fig. 11 shows a condition in which the lacunae have increased to such an extent as to extend beyond the chromatin, and the contained karyolymph is therefore directly exposed to the cytoplasm in these places. The result here, as shown in *fig. 11*, is an incomplete membrane surrounding each daughter nucleus, with the membrane only visible in those regions where the cytoplasm is exposed to the karyolymph.

From the above observations it would seem that as the chromatin of the daughter nucleus divides up in its preparation to pass into the spirem condition there is secreted a watery fluid commonly recognized as the karyolymph or nuclear sap. The karyolymph is first contained within the chromatin mass, but its volume increases and extends beyond the chromatin and consequently comes in contact with the cytoplasm. There seems little question that it is the exposure to the cytoplasm which causes the formation of a membrane around the nucleus, and the nuclear membrane is therefore a cytoplasmic structure.

GENERAL CONCLUSIONS.

It is quite natural to suppose that the secret of the origin of the cell nucleus can only be revealed by a study of the most primitive types of life. That such a supposition has influenced many investigators becomes quite evident when one glances over the enormous amount of literature that has accumulated on the cytology of the Bacteria and the Cyanophyceae. On these primitive forms a great deal of work has been done with the hope of throwing light on the origin of the nucleus. Through the efforts of Bütchli (1890, 1892, 1896), Fischer (1891, 1897), Zacharias (1887, 1890, 1897), Pella (1894), Macallum (1895, 1900), and many others, much valuable data has been accumulated, but as yet no satisfactory conclusions have been reached. This failure is probably due to the fact that many investigators have not a correct morphological conception of the nucleus as we find it in the higher types of life.

As a result of the above studies on the formation of the nuclear membrane, the writer's conception of a nucleus has been considerably modified. Instead of the nucleus being regarded as a sac containing karyolymph, chromatin, nucleoli, linin, and a membrane, the chromatin alone is regarded as the only permanent morphological constituent of the nucleus. The karyolymph and the membrane are merely temporary physiological results. The accumulation of the nuclear sap is the result of protoplasmic activity. It is first secreted within the chromatin mass and later surrounds it. The cytoplasm coming in contact with the nuclear sap forms a membrane in precisely the same manner as it forms the tonoplast when it comes in contact with the cell sap of the vacuole. The writer therefore regards the nuclear sap as no more permanent than the cell sap, and the nuclear membrane as no more permanent than the tonoplast. Furthermore, as we have shown that the nuclear membrane is of cytoplasmic origin, this structure is to be regarded more as the inner limiting layer of cytoplasm than as a constituent of the nucleus.

To convince one's self that the chromatin is the only permanent element of the nucleus, it is only necessary to observe the

various stages of mitosis. Immediately after the nucleus divides there are two masses of chromatin in the cell. For some time these masses of chromatin are surrounded by neither karyolymph or membrane, and yet no one will deny that they are nuclei. The law that the nucleus never arises *de novo*, but always from the division of a preexisting nucleus, does not hold good if we regard the membrane, karyolymph, or nucleoli as morphological elements. We must either change this law of the permanency of the nucleus or change our morphological conception of this organ. As there seems to be no doubt as to the permanency of the chromatin from cell to cell, and as the other constituents of the mature nucleus are physiological results which are entirely renewed with each succeeding mitosis, there is good reason to regard the chromatin alone as the nucleus, whether it is surrounded by karyolymph and membrane or lying freely in the cytoplasm.

The question as to the existence of a nucleus in the Cyanophyceae and Bacteria has been the subject of controversy for a great many years and even up to the present time it has not been satisfactorily shown that such an organ exists in the cells of these primitive organisms. Among the modern workers in this field, as Zukal (1892), Palla (1894), Nadson (1895), Bütchli (1892-1896), Zacharias (1897), Fischer (1897), and Macallum (1895–1900), a great divergence of opinion is expressed. Some claim that a nucleus is present; while on the other hand many claim that no structure exists that can be interpreted as a nucleus: others again have shown quite conclusively that chromatin-like granules are present, but hold that these do not represent the nucleus. Of the writers who hold this latter view Macallum (1900) expresses himself probably more strongly than the others. In his work "On the cytology of non-nucleated organisms" he finds that the cells of Beggiatoa and of the Cyanophyceae contain granules which stain like chromatin and give all its known reactions. Although he regards these structures analogous to chromatin, he concludes that "there is no nucleus nor any structure which resembles a nucleus in the Cyanophyceae." He holds a similar view in regard to the Beggiatoa cell

As the highly complex nature of the typical nucleus has doubtless been the result of the demand for a physiological division of labor in the cell, there is no reason why we should expect to find such a condition in the primitive cell of the Cyanophyceae or Bacteria. The majority of the writers who have investigated the cytology of these forms agree that chromatin-like granules exist in these primitive cells, but that these granules are surrounded by neither nuclear sap nor membrane, and many therefore do not regard these granules as representing the nucleus. As I have attempted to show that the so-called nuclear membrane is merely the inner limiting membrane of cytoplasm, and that every typical cell passes through a phase in its history when it consists of nothing but chromatin granules, the writer sees in this a possible reconciliation for the divergence of opinion that is held in regard to the nucleus of the Cyanophyceae. For might we not regard that stage of the daughter nucleus when it is devoid of a membrane as representing a phase in its ontogeny which approaches that primitive condition which we find in the cells of the bacteria and Cyanophyceae, although the chromatin-like bodies in the cells of these forms may not be the ancestral nucleus.

In conclusion, I take pleasure in acknowledging my indebtedness to Dr. Bradley M. Davis and Dr. Charles J. Chamberlin for advice, and to the Botanical Department of the University of Chicago for the excellent facilities for cytological research which were placed at my disposal.

SUMMARY.

The results of the above observations may be briefly summarized as follows:

The typical nucleus of the higher plants is a water cavity structurally similar to that of the cell vacuole.

The chromatin is the only permanent constituent of the nucleus. The karyolymph, linin, nucleoli, and membrane are renewed with each succeeding mitosis.

The nuclear membrane originates like the tonoplast. It is formed by the cytoplasm coming in contact with the karyo-

lymph just as the tonoplast is formed by the cytoplasm coming in contact with the cell sap.

The karyolymph is no more permanent than the cell sap, and the nuclear membrane is no more permanent than the tonoplast.

As the nuclear membrane is of cytoplasmic origin, it is regarded as the inner limiting membrane of cytoplasm rather than as a constituent of the nucleus.

Although the chromatin granules found in the cells of the Cyanophyceae and Bacteria are surrounded by neither karyolymph nor membrane, these granules nevertheless represent the nucleus, since every highly organized nucleus passes through a stage in its development when it consists of nothing but chromatin.

It is further suggested that the primitive nucleus probably did not secrete a karyolymph and therefore no nuclear membrane was formed.

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EXPLANATION OF PLATE XV.

The figures were drawn with the aid of Abbe's camera lucida, Zeiss homogeneous immersion, objective $\frac{1}{12}$, apert. 1.25, compensating ocular no. 8.

FIG. 1. A portion of a pollen mother-cell of *Passiflora coerulea* during nuclear division; the figure shows the chromosomes of one daughter nucleus at the poles; the connective fibers are still attached to the chromosomes but otherwise the latter lie freely in the cytoplasm.

FIG. 2. A later stage of the same; the chromosomes have fused together and the daughter nucleus is represented as a large single irregularly shaped mass of chromatin.

FIG. 3. Shows the presence of small lacunae within the chromatin mass of the daughter nucleus; these lacunae are the first indications of the accumulation of the nuclear sap or karyolymph.

FIG. 4. Shows an increase in the amount of karyolymph; the smaller lacunae have united, forming one central cavity and the chromatin has the appearance of a sphere with the karyolymph in the center; in section the daughter nucleus has the appearance of a ring of chromatin surrounding a central cavity of karyolymph.

FIG. 5. Shows a somewhat later stage than *fig. 4;* the karyolymph has increased in quantity and the chromatin has undergone still further modifications in its shape; up to this stage no trace of a nuclear membrane could be detected; the chromatin lies freely in the cytoplasm.

FIG. 6. This shows a stage in the daughter nucleus where the karyolymph has increased to such an extent and the chromatin has become so divided that the latter is no longer completely surrounded by cytoplasm; as a result the cytoplasm is for the first time exposed to the karyolymph; at the region

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of contact of the karyolymph with the cytoplasm we can observe the first indication of the nuclear membrane.

FIG. 7. Shows a still later stage where the karyolymph has increased considerably and the chromatin has become divided to such an extent as to expose the cytoplasm to the karyolymph on all sides, and as a result we have a complete membrane formed; the daughter nucleus is now perfectly spherical, containing all the elements of a mature nucleus.

FIG. 8. Shows a daughter nucleus with the chromatin in the spirem stage; this is after the cell plate has been formed and the accumulation of the granular zone around the daughter nucleus indicates that it will soon prepare for the second division.

FIG. 9. An archesporial cell of *Equisetum limosum;* the chromosomes have fused together and each daughter nucleus consists of a large irregularly shaped mass of chromatin.

FIG. 10. The same at a later stage, showing several lacunae containing karyolymph within the chromatin mass of each daughter nucleus.

FIG. 11. A still later stage showing an increase in size and number of the lacunae; some of the latter have increased to such a size as to extend beyond the chromatin, and as a result the cytoplasm becomes exposed to the karyolymph; the result is the appearance of a membrane at the region of contact of the cytoplasm with the karyolymph.

FIG. 12. Shows a stage where the volume of karyolymph has increased to such an extent as to completely surround the chromatin; the cytoplasm comes in contact with the karyolymph on sides resulting in the formation of a complete membrane about each daughter nucleus.

FIG. 13. Showing the vacuolar appearance of the mature nucleus of the archesporial cell of Equisetum; it also shows the relative size of these nuclei to the cytoplasm.

OOGENESIS IN SAPROLEGNIA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XLVI.

BRADLEY MOORE DAVIS.

(*Concluded from p. 249*)

THEORETICAL CONSIDERATIONS.

THE writer has once before (Davis, 1900) treated a number of topics suggested by recent studies on the Phycomycetes. The advances in this field of research, and also among the Ascomycetes, have been significant, and we seem to be nearing a point where much clearer conceptions of morphology and phylogeny may result. In this paper we shall take up a number of considerations suggested by this and other investigations since 1900, and for convenience they will be grouped under headings as follows:

1. Homologies of the coenogamete.
2. Origin and evolution of the coenogamete.
3. Pyronema and coenogametes among the Ascomycetes.
4. Phylogeny of Phycomycetes and Ascomycetes.
5. The nucleus of Phycomycetes in ontogeny.

HOMOLOGIES OF THE COENOGAMETE.

The writer suggested the term "coenogamete" (Davis, 1900) as appropriate to fusing multinucleate masses of protoplasm whose individual nuclei are actually or potentially sexual. Stevens's first paper (1899) on *Albugo Bliti* really opened the field in its newer cytological aspects. Since then Harper (1900) has described for the ascomycete Pyronema strikingly similar conditions, as has also Juel (1902) for Dipodascus; and from the studies of Gruber (1901) we know more about the sexual processes in the Mucorales. Harper's results will be considered in a special connection. It is important at the outset that we understand clearly the homologies of the coenogamete.

Are all coenogametes homologous with one another, and from

what have they been derived among the algae? It will be agreed that the Mucorales, *Albugo Bliti*, and Pyronema illustrate completely the conception of a coenogamete. It is a part of our problem to determine the relation of these conditions to the sexual organs in other species of Albugo, and in Peronospora, Pythium, and the Saprolegniales. There may be some hesitancy in following the series of homologies that the writer will propose, and the evolutionary history to be suggested; but he can see only two possibilities, of which one is so obscure that it seems almost impossible in the light of our present knowledge, incomplete as it is.

The most important structures in the coenogamete are the nuclei, and there can be hardly any question that they individually stand for energids, which among the algae are independent uninucleate gametes. Stevens's (1899) term "compound oosphere" expressed very well this conception of the conditions in *Albugo Bliti*. It was employed when this form was the only type known presenting the structure implied by the phrase, and these conditions might have been purely exceptional. But we now know from later studies of Stevens (1901) that other species of Albugo, *A. Portulacae*, *A. Tragopogonis*, and *A. candida*, have phases of ontogeny identical with the essential periods of oogenesis in *A. Bliti*, and may be brought into very intimate relation to the latter species. We also know that the coenogamete is not restricted to the Peronosporales, but is characteristic of the Mucorales, and is found also among the Ascomycetes. It is not likely that we shall retain the phrase "compound oosphere," for a broader conception will probably take its place, but a purpose has been served and a field opened to investigation that was quite undreamed of by the earlier investigators of the Phycomycetes.

The nuclei of coenogametes are homologous with nuclei in a gametangium destined to develop independent sexual cells. Hartog's (1891) conception of the nuclei in the periplasm of Peronospora as representing degenerate gametes has been completely justified, and there are very good reasons for believing that the nuclear divisions in the oogonium and antheridium of

the Saprolegniales and Peronosporales are "phylogenetic reminiscences of the formation of gametes." The attempts to establish special functions for these mitoses as reduction divisions for the eggs have been unsuccessful.

The oogonia and the antheridia of the Peronosporales, Saprolegniales, and Pyronema are the homologues of gametangia, and consequently of that simplest type of coenogamete, as illustrated in the Mucorales. There is everything in the morphology of these structures to favor these conclusions, but only recently have we known the details of protoplasmic organization. When an entire gametangium functions as a gamete, as in the Mucorales, it becomes a coenogamete. In Pyronema, Albugo, and the multinucleate eggs of the Saprolegniales the coenogametes are restricted portions of the protoplasm in such gametangia, but it is obvious that in Pyronema and Albugo, the gametangium behaves as a whole in a manner strictly similar to the fusion of the coenogametes in the Mucorales. It should be noted that these homologies are quite independent of the problem of the origin of the coenogametes in the various groups. That topic will be treated in the next section of the paper.

Stevens (1901) has carried the homologies a step farther in suggesting that the receptive papilla from the oogonium of the Peronosporales marks the position of the pore that develops in the gametangia of algae to give entrance or exit to the sexual elements. This is a very interesting comparison and is worth following to its limits. Thus the points of fusion of the coenogametes of a mold may be homologous with the points of exit of the motile gametes from the gametangium of some algal ancestor.

The term "coenogamete" should be employed in the strict sense indicated when the term was proposed (Davis, 1900, p. 307). It is a structure containing more than one gamete nucleus, and generally very many functional or potential gamete nuclei. It is generally homologous with a gametangium, the binucleate and trinucleate eggs of the Saprolegniales and the multinucleate eggs of *Sphaeroplea annulina Braunii* (Klebahn, 1899) presenting the only exceptions, for the oogonia of *Albugo*

Bliti, *A. Portulacae*, and *A. Tragopogonis* really act as a whole, and it is hardly possible to separate in these forms the coenogametes (oospheres) from the gametangia. When we say that the oogonium of Albugo, Peronospora, Sclerospora, and Pythium acts as a whole, we mean that the periplasm is not to be considered as waste material, but as a specialized region of the cell, with important functions in relation to the eggs, which it helps to protect by assisting in the formation of heavy walls. The Mucorales, Pyronema, and these three species of Albugo furnish the best known illustrations of coenogametes.

ORIGIN AND EVOLUTION OF THE COENOGAMETE.

There seem to be only two possible sources of the coenogamete. It is conceivable that a uninucleate sexual element might become multinucleate, perhaps through such an increase in the protoplasmic content that more than one nucleus would be required to control satisfactorily its activities. The second possibility is an origin from a multinucleate gametangium that has given up the production of uninucleate gametes, and acting as a unit becomes itself a sexual organ, a coenogamete. Such an evolutionary process would find its analogy in those sporangia (conidia) of certain species of Pythium and Peronospora, which now germinate as a whole (by a tube) instead of forming zoospores.

The first possibility has absolutely no evidence in its support. There is no series of forms whose sexual cells pass from a uninucleate condition to a multinucleate. There are no indications that such an evolutionary process has ever taken place among plants. There are only two instances known where eggs, free from periplasm, are multinucleate. The eggs of Albugo are so intimately associated with periplasm that they cannot be considered apart from the gametangium in which they lie. These two examples are the binucleate and trinucleate eggs of the Saprolegniales and the multinucleate eggs of *Sphaeroplea annulina Braunii*. Our investigations of Saprolegnia have shown that the processes of oogenesis in that group have as an end the sacrifice rather than the preservation of nuclei, and the uninucleate condition is evidently the goal of evolution. Klebahn's (1899) and

Golenkin's (1899) studies of Sphaeroplea are incomplete in certain cytological details of oogenesis, and the fact that the eggs of some forms are uninucleate suggests caution before laying emphasis on the multinucleate condition. It is possible that further study will relate the multinucleate eggs to the uninucleate, as in Saprolegnia.

What evidence have we of the second possibility, *i. e.*, the origin of the coenogamete from a multinucleate gametangium which, ceasing to form uninucleate sexual cells becomes itself a coenocytic gamete? Most important is the exceedingly interesting series of four species of Albugo described with so much detail by Stevens (1901). We cannot take up this investigation except to notice that the four species form a well-graded series in which the evolutionary direction is clear and very important for the conclusions that we are striving to establish. The oospheres of *Albugo Bliti* and *A. Portulacae* contain many functional gamete nuclei, that of *A. Tragopogonis* several potential and several functional, and that of *A. candida* several potential and one functional. In this series the coenocentrum is very small in *A. Bliti* and *A. Portulacae*, larger in *A. Tragopogonis*, and very large and strongly chemotactic in *A. candida*. A fifth form has been added to this series by Ruhland (1902), who finds that *Albugo Lepigoni* is even more highly specialized than *Albugo candida*, since it contains an extraordinarily large coenocentrum. The evolution in complexity is plainly from *A. Bliti* to *A. candida* and *A. Lepigoni*, that is, from the multinucleate egg to the uninucleate. And this series offers the most striking evidence against the evolutionary possibility considered in the previous paragraph.

Now, the multinucleate eggs of Albugo are not the most primitive types of coenogametes, because they contain only a portion of the total number of the nuclei in the gametangium, many of the sister nuclei passing into the periplasm. They are not as simple as the coenogametes of the Mucorales, nor yet as primitive as the oogonium of Pyronema, which has no periplasm, although it sacrifices a large number of nuclei in the conjugating tube (trichogyne), and by this specialization presents conditions more complex than the molds.

It is the specialization of a periplasm simultaneous with the reduction in the number of functional gamete nuclei that has made possible the elaborately organized oogonium of Peronospora, Sclerospora, Albugo, Araiospora, and to a lesser degree Pythium. And the coenocentrum is perhaps most largely responsible for the highest specialization. The coenocentrum largely influences and perhaps controls the position and structure of the eggs. The larger the coenocentrum, the more direct is the effect on neighboring nuclei, and the greater is the benefit to such nuclei as are so fortunate as to be within its sphere of operations. So in the struggle for existence among potential gamete nuclei in the oogonium, the coenocentrum has a power of assistance that, according to its degree of development, determines the structure of the egg, whether multinucleate or uninucleate. The evolutionary trend is physiologically precisely the same as is shown among the algae (Fucales, Vaucheria), when potential gamete nuclei are sacrificed to provide functional nuclei with a large amount of richly nourished protoplasm.

But it should be noted that, although the evolutionary processes in the Peronosporales have resulted in uninucleate eggs, these structures are not strictly homologous with the eggs of algae. They are homologous only in the sense that the eggs of Volvox, Fucus, Vaucheria, Chara, and several other highly developed algae are homologous. In these algae the eggs have an ancestry from much simpler types of gametes, and relationships must be traced through these or perhaps through older forms of asexual spores. The oogonium of the higher Peronosporales has come through a series of coenogametes of which *Albugo Bliti* represents a certain stage, but whose earlier forms must have been simpler. The primitive conditions probably had a structure comparable to the coenogametes of the Mucorales, and that type of structure finds its nearest approach among the algae in the gametangia that discharge numerous gametes, as illustrated by Cladophora and many of the Siphonales.

But it will immediately be asked: What are we to do with such algal types as Vaucheria, Sphaeroplea, Oedogonium, etc.? Have they no relation to the fungi? This will be considered

under the topic "Phylogeny of the Phycomycetes and Ascomycetes." It is important that we emphasize now the evolutionary process brought out by Stevens's work on the four species of Albugo, and extend the results of that study to the Penorosporales as a whole. Accordingly we have good reason to believe that the uninucleate eggs of *Albugo candida*, Peronospora, and Pythium have not been derived from the eggs of algal ancestry, but from coenogametes which passed through the stage illustrated by *Albugo Bliti*, and came from much simpler conditions, probably resembling in many respects the coenogametes of the molds and Pyronema.

An origin of the simplest types of coenogametes (molds and Pyronema) from gametangia of algae presents certain difficulties that should be discussed. The process would involve a change in the activities of a structure from one where the nuclei show a considerable degree of independence to one in which the nuclei cooperate in a coenocytic cell that acts as a unit. An evolutionary process comparable to the above must have taken place with the development of the multinucleate zoospore of Vaucheria, if its nuclei stand for the numerous zoospores generally formed in the terminal sporangia of the Siphonales. And a similar evolution, as has been mentioned before, is shown in the development among the Peronosporales of conidia (which germinate by tubes) from sporangia (conidia) that form zoospores. Such conidia and the zoospores of Vaucheria are not considered the equivalent of tissues, but units in their physiological behavior, just like uninucleate spores.

Similarly, the coenogamete is not the equivalent of a tissue, and must not be considered as made up of independent gametes associated together because their cytoplasm is fused into a common mass. It exhibits the same sort of individuality as any coenocytic cell or structure. We no longer draw sharp lines between uninucleate and multinucleate cells, for we realize that the transformation of the first into the second is a very simple matter, and that the unity of the coenocyte is not disturbed by its having several or many nuclei, for these do not occupy fixed positions in the cell but wander with the varying movements of the

protoplasm. The coenogamete is as much an individual cell as the uninucleate gamete, and distinctions can no more be drawn between these two structures than between the adjacent uninucleate and multinucleate cells of many plants (Chara, Rhodophyceae, etc.). In view of its structure and behavior the term "coenogamete" seems to the writer appropriate.

PYRONEMA AND COENOGAMETES AMONG THE ASCOMYCETES.

Harper's (1900) investigation of Pyronema has established a condition in the Ascomycetes very similar to that among the Phycomycetes. Pyronema has as conspicuous a coenogamete as the Mucorales or *Albugo Bliti*. Its peculiarities do not affect the essential cytological structure of the fusing multinucleate masses of protoplasm whose gamete nuclei unite in pairs, as in *Albugo Bliti* and probably in the molds. The sexual apparatus of Pyronema differs from the molds chiefly in the development of that specialized structure the conjugating tube (trichogyne). This organ is manifestly of advantage because it effects a union with the antheridium, and probably, as Harper suggests, represents the same sort of outgrowth from a sexual element as a trychogyne. The nuclei in the conjugating tube break down, and the structure finally becomes merely the channel through which the protoplasm from the antheridium flows into the oogonium. This movement of the protoplasm is very similar to *Albugo Bliti*, and the resemblance is carried still farther in the distribution and fusion of the gamete nuclei in pairs throughout the oogonium. The "receptive papilla" of Albugo is developed from the oogonium, and the conjugating tube in Pyronema may be considered an elaboration of such a growth tendency. Periplasm is lacking in Pyronema, but the mass of nucleate protoplasm that passes into the conjugating tube may relieve the oogonium of those conditions that result in the extensive degeneration of potential gamete nuclei in Saprolegnia or the somewhat similar conditions affected by the differentiation of a periplasm in the Peronosporales.

It is not to be supposed that the coenogametes of Pyronema are closely related to those of the Mucorales or the Peronosporales, excepting as all of these structures are the homologues

of gametangia. But it is important that we should recognize this condition among the Ascomycetes as one that further study may show to be not uncommon in the group. Juel (1902) reports it for Dipodascus. Miss Nichols's (1896) studies on Ceratostoma, while inconclusive in cytological details, are of importance in this connection. She has described and figured multinucleate oogonia (archicarps) and antheridia, which are said to fuse. They are apparently coenogametes, and it is probable that these structures will be found in other genera of the Pyrenomycetes and Discomycetes. There are several forms whose archicarps suggest a coenocytic structure (Eremascus, Ascobolus, Sordaria, Eurotium, etc.).

The student of the homologies and evolution of the sexual organs among the Ascomycetes now finds himself face to face with the same problem that has been presented to Stevens and myself for the Phycomycetes. What is the relation of the uninucleate gamete (*e.g.*, Sphaerotheca) to the multinucleate? Which condition is the more primitive?

There is likely to be some confusion of homologies among the sexual organs of the Ascomycetes. The oogonium (archicarp) of Sphaerotheca is morphologically a gametangium, and so is the antheridium, but both structures are physiologically gametes. The oogonium and antheridium of Pyronema are morphologically gametangia, so that in comparing these two forms we are dealing with homologous structures. Of course, we use them merely as illustrating certain sexual conditions; indeed, they are almost the only Ascomycetes whose sexual organs have been thoroughly studied, with the exception of certain lichens and the Laboulbeniaceae in which the conditions are very different and will be considered later.

The problem then will be: Did the uninucleate condition of the gametangium, as represented by Sphaerotheca, come from a multinucleate gametangium (coenogamete) illustrated by Pyronema, or is it the progenitor of the latter? We have no series of forms in the Ascomycetes such as the four species of Albugo studied by Stevens to help us to a conclusion. But the problem in the Ascomycetes seems to be identical with that of the

Phycomycetes discussed in the previous sections of this paper. To derive a multinucleate gamete (Pyronema) from a uninucleate (Sphaerotheca) involves an evolutionary process quite unknown to botany. To derive a uninucleate gamete from a coenogamete merely demands a gradual reduction of the number of gamete nuclei, a process which we know to have taken place in several groups of algae independently of one another, and one which is beautifully shown in Stevens's series of four species of Albugo.

Harper (1900, pp. 388, 389) seems to be undecided as to the developmental relation of conditions in Sphaerotheca to such as are presented in Pyronema. He shows that the oogonium of Sphaerotheca could easily be given the form of Pyronema by the development of the beak into a conjugating tube with some minor changes in the position of the antheridium. But he disregards the internal changes necessary to derive a coenogamete from a uninucleate gamete. And at the end of the same paragraph he says: "Still I am inclined to believe that the reverse process has taken place and that the sexual apparatus with the trichogyne represents the more primitive type for the Ascomycetes." To the writer resemblances of form have very little value in such comparisons, and relationships must be traced through agreement in the details of protoplasmic activities. And again, as Harper points out, the general morphology of the Erysipheae is much higher than that of Pyronema. But Professor Harper by his last statement has warded off criticism, and perhaps, with the evidence from Albugo and Saprolegnia before him, he will feel more certain, with the writer, that the coenogamete when related to the uninucleate gamete always represents more primitive conditions.

And this conception has a very interesting relation to the possibility of deriving the trichogynes of lichens and the Laboulbeniaceae from a primitive type of sexual organ that may have been a coenogamete. Of course, there is no reason why a uninucleate gamete (archicarp) among the fungi might not develop a simple trichogyne, as has been done in the Rhodophyceae, but the trichogynes of the lichens and the Laboulbeniaceae are generally systems of cells quite distinct from the female

gamete (carpogenic cell). These conditions are nowhere presented in the red algae, and it is very difficult to understand how a uninucleate gamete could develop such elaborate structures. But taking the suggestion of Harper (1900) that the conjugating tube of Pyronema is an outgrowth similar to a trichogyne, there are presented possibilities of various elaborate structural developments, because the outgrowth has so much protoplasm and many nuclei to draw upon. The evolutionary tendency of a coenogamete is to reduce the number of functional gamete nuclei, generally by the sacrifice of many, but these with accompanying cytoplasm are sometimes employed to advantage in developing structural adaptations. In the Peronosporales the advantage lies in the activities displayed by the periplasm in assisting to form the spore wall. Araiospora (Thaxter, 1896) utilizes the periplasm to develop a cellular envelope surrounding the egg. The conjugating tube of Pyronema is evidently a desirable specialization, insuring a union with the male organ. Perhaps the elaborate multicellular trichogyne is the result of similar activities on the part of archicarps that are or were coenogametes.

It is obvious that this possibility has very important relations to comparisons that have been made between the trichogynes of the Ascomycetes and those of the Rhodophyceae. It is not easy to homologize these structures, and it is difficult to conceive the evolution of any group of the Ascomycetes from the red algae. The Laboulbeniaceae exhibit certain strong resemblances in a general similarity of cell structure, but peculiarities confront one whenever the comparison is carried into details. Nevertheless a relation of this group to the Rhodophyceae remains a possibility, although it can hardly be more than mere speculation until we have much greater cytological knowledge of sexual processes here and in other Ascomycetes.

But the coenogamete may be found widespread among the Ascomycetes, which suggests a new point of view that is worth attention. It is possible that the coenogamete may come to be recognized as a primitive type of sexual organ in the Ascomycetes, as the writer believes it to be for certain groups of the

Phycomycetes (Mucorales, Saprolegniales, and Peronosporales). Perhaps the complex conditions of such highly specialized groups as the Erysipheae, lichens, and Laboulbeniaceae may be related to the peculiar activities and possibilities of diverse development in this interesting sexual cell, the coenogamete. Sphaerotheca may readily stand as the last step in a process of nuclear reduction. Pyronema certainly exhibits the tendency to utilize superfluous nuclei and protoplasm in developing that advantageous structure, the conjugating tube. And possibly such tendencies might result in the production of the elaborate trichogynes of the lichens and Laboulbeniaceae, and in the latter group the structure that resembles the procarp of the red algae.

PHYLOGENY OF THE PHYCOMYCETES AND ASCOMYCETES.

The reader has probably already noted that some standpoints have been taken at variance with the generally accepted ideas of relationship among the Phycomycetes and Ascomycetes, and of these groups to an algal ancestry. A protest is sure to be offered against the disregard of certain Phycomycetes and algae in the attempt to derive the Mucorales, Saprolegniales, and Peronosporales from an ancestry with coenogametes.

There are some Phycomycetes that are much closer to the algae than any of the groups mentioned above. Monoblepharis and Myrioblepharis (Thaxter, 1895) exhibit sexual organs, zoospores, and vegetative structure with striking resemblances in various particulars to such algae as Vaucheria, Oedogonium, and Sphaeroplea. The homologies can hardly be questioned and will not be elaborated here. These fungi, and possibly some of the Leptomitaceae, seem to be close to heterogamous (oosporic) algae and may well have come from that region of the Thallophytes. The family Leptomitaceae includes some very remarkably types which have been well described by Thaxter (1896). Their position must remain somewhat uncertain until we know the nuclear structure of the sexual organs, but the general morphology of some forms indicates a relationship to the Peronosporales. Araiospora (Thaxter, 1896) is likely to prove especially interesting as illustrating an activity of the periplasm in forming a cellular envelope around the oospore, that is not

shown in any other type and has important bearings on the possible development of tissues of considerable complexity by the coenogamete.

But many difficulties present themselves when the Monoblepharidae are made a starting-point for a line of ascent to the Peronosporales, as is done by Trow (1901, pp. 306, 307) when he arranges in series Monoblepharis, Saprolegnia, Pythium, and Albugo (Cystopus). These forms are not so similar that close relationships are manifest either through morphology or ontogeny. Under the most favorable interpretation it must be granted that they are at present widely divergent and highly specialized types, even assuming that ancestral forms now extinct might have had more general characters. Such speculations are, of course, entirely justifiable, if they do no violence to developmental history.

However, as has been shown, such an evolution must assume either that uninucleate gametes became multinucleate or that differentiated eggs (Monoblepharis) lost their high state of specialization and finally their entire individuality in the coenogamete of the Peronosporales. Both processes are opposed to what we know has been the evolutionary history of sexual cells in several divergent and independent groups of algae. We are called upon to accept a "subjective phylogeny" opposed to well-established cytological processes.

The situation is somewhat similar to that presented to the Brefeldian school with respect to the origin of the ascus from the sporangium of a mold. Harper has shown that the protoplasmic activities of sporogenesis in the sporangium and ascus are along entirely different lines with nothing in common. To the writer such differences in cytological processes completely outweigh conclusions from any series of types presented on a basis of general form resemblance. Form resemblance between the ascus and sporangium can have very little morphological value until it be accompanied by evidence satisfactorily explaining the differences of protoplasmic organization and behavior. And the elaborate phylogenetic structure built by Brefeld and his followers is sadly in need of a foundation, if not already a

ruin. Form resemblance must be in complete harmony with cytological conditions to have weight.

Trow (1901) has criticised a developmental line that the writer indicated in 1900, which, he states, is an attempt to derive Oomycetes from a zygomycetous ancestry, and which he considers an example of "subjective phylogeny." I have carefully examined what was written in that paper (Davis, 1900, pp. 304–9), and, not finding any reference to specific phylogenetic ancestry, am compelled to suggest to Trow a more careful reading and citation of that article. I presented there suggestions for the developmental history of the sexual conditions in the Peronosporales from coenogametes derived from the gametangia of algae. These coenogametes at a certain stage in the process of sexual differentiation would be similar to the sexual organs of the Mucorales. The molds were used to illustrate a well-defined sexual condition, which is not at all suggesting that they are the ancestors of the Peronosporales (Oomycetes).

But the present investigation of Saprolegnia, together with Stevens's (1901) later studies on Albugo, have strengthened my faith in the suggestions of that former paper (Davis, 1900). The Mucorales, Saprolegniales, and Peronosporales are generally acknowledged to be closely related groups, but it seems probable that the affinities are only through the somewhat similar conditions of sexual organs derived from the coenogametes of some common ancestry. There are many peculiarities of life-habits, life-histories, and methods of asexual reproduction. Of these three groups the Mucorales present the simplest conditions of sexuality and illustrate most nearly the structure of the primitive coenogamete. The Peronosporales and Saprolegniales are difficult to relate to one another, for the higher development of the coenogamete is apparently progressing along divergent lines. In the Peronosporales the protoplasmic differentiation in the oogonium determines a centrally placed egg in an enveloping periplasm, for a single coenocentrum dominates the process of oogenesis. In the Saprolegniales the ooplasm gathers by cleavage around a number of coenocentra, and all the protoplasm passes into the resulting eggs. To the writer the second process seems

less highly specialized than the first and the Saprolegniales lower than the Peronosporales with respect to sexual processes. But oogenesis in these two groups shows such marked differences in their evolutionary tendencies that the question of the relative level of each process has very little import.

The Saprolegniales are more difficult to understand in relation to a coenogamete ancestry than the Peronosporales, because the many eggs without periplasm suggest at once the stage in heterogamy illustrated by Sphaeroplea. However, the processes of oogenesis are probably very different in the two types. The egg origins of Saprolegnia have a great many potential gamete nuclei, and that stage indicates strongly the coenogamete ancestry. By numerical reduction of the gamete nuclei the egg of Saprolegnia has proceeded to a point where it has almost ceased to be a coenogamete, that condition only being presented in the bi- and trinucleate eggs.

It will be difficult for many to give up the idea that Vaucheria is not a suitable starting point for the line of higher Phycomycetes. The chief objection is the incompatibility of the processes of oogenesis where relationship demands agreement even in the details of cytology. We have only the accounts of Oltmanns (1895), Behrens (1890), and Klebahn (1892, p. 237), which are not in complete agreement on some important points, and perhaps further study may reveal conditions that are only suspected. In considerations of this sort it is important to know the relation that Vaucheria bears to the algae as a whole. Although generally classed among the Siphonales, Vaucheria has little in common with that group excepting the coenocytic thallus. It stands alone as the only heterogamous form (oosporic) in a very large assemblage characteristically isogamous. Generally presented as a type of the Siphonales, Vaucheria is not really representative of that group, which is much better illustrated by such forms as Codium, Bryopsis, or Penicillus. The affinities of Cladophora with the Siphonales are now better understood, and we see that this form except for the septate thallus — whose cells are, however, multinucleate — has all the characters of the Siphonales. It is this region of the algae (Cladophora, Codium,

etc.) that presents to the writer's mind conditions most nearly like the ancestry of the Mucorales, Saprolegniales, and Peronosporales, that is, an ancestry whose sexual organs were coenogametes. Perhaps, however, further studies on the oogonium of Vaucheria may bring this structure into harmony with coenogametes.

The coenogamete among the fungi must have come through the homologous structure among the algae, the gametangium. We cannot suppose that such gametangia were highly specialized. It is hardly possible that they were heterogamous, for a highly differentiated oogonium would not be likely to return to conditions as simple as the primitive coenogamete. The gametangia of such isogamous algae as Cladophora and Codium present most nearly the structure demanded of the progenitors of the primitive coenogamete, but, of course, these forms are mentioned only as illustrations of conditions undoubtedly present in many groups of algae at various periods in their evolutionary history.

We can only speculate as to the manner in which a gametangium might become a coenogamete. The writer has already offered some suggestions on this point (Davis, 1900, p. 308), and he is more inclined to them since the recent studies of Harper (1900) and Stevens (1901) and the present investigation of Saprolegnia. We can readily conceive the derivation from isogamous algae of groups of aquatic fungi with terminal sporangia discharging motile gametes after the manner of Cladophora. Should such fungi leave the water and adopt terrestrial habits either as saprophytes (Mucorales) or as parasites (Peronosporales), certain changes in the sexual processes would be very likely to result. The gametangia could not form and discharge motile gametes excepting when wet, and would be compelled to adapt themselves to the aerial environment. They would be very likely to develop such unity of structure and behavior as is displayed in Pythium and Peronospora by those sporangia (conidia) which have given up the habit of forming zoospores and now germinate by a tube. The gametangium would become a coenocytic unit, with the chemotactic qualities and possibilities associated with sexuality. These chemotactic influences

might be satisfied by the fusion of the gametangia (coenogametes) in pairs whereby the gamete nuclei would be able to unite two by two in a common protoplasmic medium. This process would take the place of the conjugation of motile gametes in water, and apparently satisfy all the hereditary demands as far as nuclei are concerned. The structure resulting from the fusion of these simplest coenogametes would be very similar to the zygospore of the molds.

Although there are no coenogametes among the algae, the sexual processes in the Conjugales have some features worth noting in this connection. In the desmids the gametes slip from the parent cells and fuse as naked masses of protoplasm. But in the filamentous Zygnemaceae and Mesocarpaceae the energids (gametes) remain in the respective parent cells which push out conjugating processes. The conjugating processes are surrounded by a cell wall so they are in every respect similar to the conjugating tube of Pyronema or the receptive papilla of the Peronosporales, excepting that they emanate from a uninucleate cell instead of a coenocyte. It is important to note that the conjugation processes in the Phycomycetes and Ascomycetes have their analogies in the algae, for it might be suggested that the development of such a structure by a coenogamete would be difficult. On the contrary, it seems the natural expression of any cell with chemotactic tendencies, whether uninucleate or multinucleate, to fuse with its neighbors. It is very probable that the development of such a conjugating tube in a coenogamete would be at the point where formerly the naked motile gametes were discharged, for that place is evidently the seat of important cytoplasmic activities.

To sum up our conception of the Phycomycetes, we must regard them as a group of several independent phyla. The Chytridiales in morphology and life-history are the lowest and resemble the algae at the level of the Protococcales. Monoblepharis, and probably several other isolated genera, seem most closely related to heterogamous algae. The Entomophthorales are too highly specialized to be easily derived directly from algal ancestry and need not be considered in this paper. There

are left the most conspicuous of the Phycomycetes in three orders, that agree primarily in having either typical coenogametes (Mucorales) or sexual organs probably derived from coenogametes (Peronosporales and Saprolegniales).

These three orders, however, can only be related to one another through a common ancestry whose sexual organs were coenogametes. The Mucorales illustrate most completely the primitive coenogamete, and for this reason in part may be considered rather the lowest of the three groups. In the Peronosporales we have an ascending series from forms such as *Albugo Bliti* and *A. Portulacae* with true coenogametes, although more highly specialized than those of the molds, to the conditions in *Albugo candida*, Peronospora, and Pythium. This advance is evidently such an evolution as would provide a single uninucleate egg with the richest supply of food and best protective walls possible. In the Saprolegniales the evolutionary trend is similar in that a great many potential gamete nuclei are sacrificed to give a uninucleate egg, but we are not yet prepared to trace exactly the steps in the origin of this oogonium. However, the probabilities are that it, too, has come from a coenogamete, and that the segmentation of this protoplasm to form many eggs does not imply a derivation from heterogamous ancestry, but special peculiarities associated perhaps with the presence of several coenocentra. Oogenesis in Saprolegnia certainly indicates an ancestry with coenogametes. The Mucorales, Peronosporales, and Saprolegniales then probably come from a somewhat similar ancestry with coenogametes, which necessitates their derivation from isogamous algae at about such a level as is illustrated today by Cladophora and forms of the isogamous Siphonales.

We do not propose to discuss the phylogeny of the Ascomycetes further than to present its problem with respect to the coenogametes. The difficulty of relating the diverse sexual organs represented by Sphaerotheca, Pyronema, the lichens, and the Laboulbeniaceae has led to suggestions that the Ascomycetes are polyphyletic. But this view has many objections in the essential unity of the ascocarps and general rhythm of the life-

histories throughout the group. Nevertheless, the various types of sexual reproduction seem very diverse when compared with one another and with conditions in the algae and other fungi.

However, should the coenogamete be established as a primitive type of sexual organ here as in the Phycomycetes, certain difficulties will be removed. The oogonium (archicarp) would be considered a development from the coenogamete along a well-established evolutionary line, that of numerical nuclear reduction. The evolutionary trend of the coenogamete would then be toward the uninucleate oogonium (Sphaerotheca) following the tendency of sexual evolution so well recognized in the algae. The groups of the Discomycetes and Pyrenomycetes would then readily arrange themselves according to the structure of the ascocarp and general vegetative complexity.

There would be left the lichens and Laboulbeniaceae, whose trichogynes at least suggest the Rhodophyceae, while in the latter group there are certain histological resemblances to this same group of algae. Granting these possible affinities, it is nevertheless very difficult to conceive the multicellular trichogyne as derived from the simple structure of the red algae. It must also be borne in mind that the structure of the ascocarp, especially among the lichens, gives no suggestion of a cystocarp, but, on the contrary, presents a structure identical with the fructification of other Ascomycetes. Were it possible for the coenogamete to develop a multicellular trichogyne (there is a multinucleate one in Pyronema) then evolutionary lines might be established that would lead very naturally into the lichens and Laboulbeniaceae. Such trichogynes would be another form of expression of this remarkable structure, the coenogamete, which is able to utilize superfluous protoplasm in such a variety of ways.

In this connection it is interesting to sum up the various ways in which the superfluous protoplasm of a coenogamete may assert itself. It may form a periplasm of importance in developing the spore wall (Peronosporales). It may form a surrounding tissue from such periplasm (Araiospora). It may develop a conjugating tube (Pyronema). And finally we suggest the possibility of

multicellular trichogynes derived from coenogametes. While this cannot be more than a speculation, nevertheless cytological and developmental investigations among the lichens and Laboulbeniaceae in relation to these possibilities are sure to bring forth interesting results.

We may then conceive the Ascomycetes as presenting two important evolutionary lines derived from a primitive coenocytic type of sexual organ (coenogamete). The first, through numerical reduction of potential gamete nuclei, results in uninucleate sexual organs (Sphaerotheca). The second line supposes the utilization of such potential gamete nuclei with cytoplasm to develop such secondary sexual structures as the conjugating tube of Pyronema and the trichogynes and procarpic apparatus of the lichens and Laboulbeniaceae.

It is difficult to relate the account of Juel (1902) for Dipodascus to conditions in other coenogametes. Juel believes that there is but one sexual nucleus in each of these multinucleate gametes, the others being "vegetative;" that there is only one fusion nucleus in the fusion cell. This gives rise to a series of nuclei around which the spores develop in the sac and the "vegetative" nuclei degenerate. The details of the nuclear activities are not reported, and many stages in the processes are completely lacking. Until we know these we must hesitate to express an opinion on the position of Dipodascus.

THE NUCLEUS OF PHYCOMYCETES IN ONTOGENY.

A detailed and complete study of the nucleus of some Phycomycetes in the various phases of ontogeny is greatly to be desired. At present we know a good deal about the nuclear activities during gametogenesis and something at the time when the oospore germinates, but the data are not complete for any one form and do not explain the most important problems of ontogeny. These concern the significance of the mitoses in the gametangia, the relative numbers of chromosomes at different periods of ontogeny, and their bearing on the sequence of generations, which is not well understood in this group.

This knowledge will demand the study of one or more types with attention to nuclear phenomena during vegetative periods,

especially at the time when asexual spores or conidia are formed, during gametogenesis, and during the mitoses following the fusion of sexual nuclei. Species of Albugo and Peronospora seem to offer the best material for these investigations. Pythium, although easy to cultivate and control, has nuclei so small that it is almost impossible to study such details. The same difficulties are met in the Mucorales and in part in the Saprolegniales, and in the latter group the complications of apogamy render the forms useless for these problems. Speculations on the reduction of chromosomes and the significance of various phases of ontogeny in this group are almost futile until we have convincing and complete data for one or more types.

Whatever may be the significance of the mitoses in the gametangia, there is no proof that they are reducing divisions, and it is probable that they are only phylogenetic reminiscences. Stevens's observations that the nuclei in the second mitosis of Albugo are much weaker in kinoplasm are interesting, but it is very questionable whether such divisions are necessary steps in the physiological differentiation of gametes. The mitosis may have simply a phylogenetic relation and the lessened kinoplasmic content be merely the result of that decrease in the size of the nuclei characteristic of advanced periods of oogenesis in these plants.

Everything seems to point to the ooplasm as trophoplasmic in character, first from the gathering of substance around the coenocentrum and second from the effect of this structure on nuclei in the vicinity. Staining reactions confirm this conclusion, but it is not wise to lay too much stress on the effect of stains in objects so small as these. And for this reason the judgment that the gamete nuclei are weak in kinoplasm must be taken with caution. The nuclei are generally smaller, and the conditions are such that the majority of them must disorganize; but the reason for this run-down state must be chiefly the general nutritive conditions of the gametangium, and not the mitoses of that period.

Stevens (1901, pp. 238, 239) lays stress on that period of oogenesis in Albugo and Perenospora termed "zonation," when

the nuclei often in mitosis move from the center of the oogonium to the periphery. He suggests "that the nuclei pass to the periphery to rid themselves of superfluous kinoplasm, possibly to prevent parthenogenetic development of the oosphere." This theory seems to the writer to suppose an order of events and degree of preformed specialization more intricate than the evidence warrants. It seems more likely that "zonation" represents an event that happens to accompany, but is secondary to, those processes which gather the ooplasm in the center of the oogonium and give the egg its coenocentrum and characteristic alveolar structure.

Indeed, the conditions that cause that extraordinary degeneration of nuclei in the oogonium must furnish in large part the solutions of these problems. This phenomenon is universal, whether the nuclei break down in the eggs themselves (Saprolegniales), or are relegated to such secondary sexual structures as periplasm (Peronosporales) or a conjugating tube, as in the ascomycete Pyronema. As we have seen in Saprolegnia, the many nuclei in the eggs during advanced stages of oogenesis are all much reduced in size, and the only thing that saves the fortunate survivors of the general severe conditions is proximity to that center of metabolic activity, the coenocentrum. There is a limit to the number of nuclei possible in a given amount of cytoplasm. The nutrition of the oogonium decreases as oogenesis proceeds, and finally reaches a point when the nuclei are sorely pressed to maintain themselves. This certainly seems to be the history for the Saprolegniales, and probably every phycomycete whose sexual organs are coenogametes, as these structures are generally formed late in ontogeny, when the period of vegetation is about completed.

The nuclei are then subjected to a keen struggle for existence, and, in spite of the fact that they are in a symplast, which is itself a unit, they may well be supposed each to look after its own interest as far as possible. The outcome of that struggle is largely determined by the activities of the cytoplasm which may develop such metabolic centers (morphologically expressed by coenocentra) that certain nuclei by good fortune of favorable

position are given great advantages over their neighbors and are finally selected as the survivors.

There are a number of instances known where sexual organs sacrifice some of their nuclei to provide the remainder with the cytoplasm at hand. Certain Fucales present notable examples, and there will probably be found other illustrations among the algae and fungi. Analogous conditions in animals have been reported, as in oogenesis of Actinosphaeria (Hertwig, 1898), and the well-known fate of supernumerary male nuclei in polyspermy. In these cases there has not been reported the same close relation between the surviving nuclei and metabolic centers of the cell as between the favored nuclei and the coenocentra of the Saprolegniales and Peronosporales. In this same connection we need more detailed accounts of oogenesis in the Fucales, Vaucheria, and Sphaeroplea.

The reasons why the oogonium overstocks itself with nuclei are probably phylogenetic and recall the time when numerous uninucleate gametes were formed from the protoplasm that now acts as a unit (coenogamete). Such uninucleate gametes were probably smaller than their homologues, the asexual zoospores, as is so characterstic of algae. Among the algae it is generally conceded that the small gamete swarm-spores result from different conditions of nourishment than their asexual homologues. It has been suggested that they are starved, but that seems a clumsy conception of very intricate processes. But there must be deep significance in the overproduction of sexual nuclei during gametogenesis and its obvious association with the deficient nutrition at the command of the gametangia. This phase of the subject has not received the attention it deserves.

SUMMARY OF THE INVESTIGATION OF SAPROLEGNIA.

OOGENESIS.

The material, *Saprolegnia mixta*, was apogamous, being entirely free from antheridial filaments.

The resting nucleus has a loose linin network and a nucleolus, and presents essentially the structure of the nucleus of higher plants.

There is one mitosis in the oogonium, the spindle being intranuclear. There are no centrosomes. The four chromosomes are derived from the linin network.

The daughter nuclei following the mitosis are much smaller than their parents. They shortly give evidence of coming degeneration, the nuclear membranes become indistinct, and the contents finally lie as granular material in clear areas resembling vacuoles.

The eggs are formed during the process of nuclear degeneration. The protoplasm in the oogonium at this period is arranged peripherally around a large central vacuole. The ooplasm collects around several centers, each of which is to become an egg origin. The egg origins are finally separated through the arrangement of vacuoles which results in the severance of connecting strands of protoplasm, and the eggs round themselves off as independent structures.

The differentiation of the egg origins takes place around a deeply stained protoplasmic body, the coenocentrum, from which delicate fibrillae radiate. The coenocentra are formed *de novo*, one for each spore origin. Each is at first a small globule, made conspicuous, however, by its fibrillar rays. It is most conspicuous in the young eggs, becoming less distinct with the ripening, and finally disappears. There is no trace of it in the oldest eggs.

The coenocentrum is a protoplasmic structure, but not a permanent organ of the cell. It is probably the morphological expression of dynamic activities in the oogonium when the egg origins are differentiated, and is a sort of focal point of the metabolic processes peculiar to oogenesis.

The coenocentrum exerts a chemotactic influence on any nuclei in its immediate vicinity. Generally one nucleus is selected and comes to lie very close to the coenocentrum, so that these two structures in the egg origins may be separated only under high magnification. This nucleus increases in size when all other nuclei in the egg origins and young eggs are degenerating, showing that it is greatly favored with respect to nourishment by its position near the coenocentrum.

Sometimes two or even three nuclei may lie sufficiently near the coenocentrum to be saved from degeneration, and such eggs are in consequence bi- or trinucleate. Binucleate eggs are not uncommon, trinucleate eggs are more rare.

As the eggs mature, the favored nucleus increases greatly in size, until it is many times larger than at the period following the mitosis. The other nuclei have generally completely disorganized, but sometimes traces remain as granules scattered in the cytoplasm.

Binucleate eggs in the Saprolegniales need have no relation to the problem of sexuality, and Trow's conclusions are not established.

SPOROGENESIS.

A general confirmation of the accounts of Rothert, Hartog, and Humphrey.

The uninucleate spore origins are differentiated by clefts that push their way from the central vacuole of the sporangium to the periphery. When the clefts reach the cell wall, the turgor of the sporangium is relieved through the escape of water, and the spore origins run together, but they soon draw apart and round themselves off as zoospores. There seem to be no cytoplasmic centers in the sporangium comparable to the coenocentra.

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NOTE.—Mr. Barker's interesting paper "The morphology and development of the ascocarp in Monascus" (Ann. of Botany 17: 167. 1903) came to my hand at the same time as the final proof of the foregoing article. I should have liked to discuss in detail a number of points which he takes up in connection with the origin and relationships of the Ascomycetes, but must defer the matter to some future time. There are some comments, however, which seem sufficiently important to justify this note. Monascus adds another form to the list of Ascomycetes whose sexual organs are coenogametes, and in so far strengthens my view that this type of sexual organ is likely to prove the primitive one for this group. I cannot agree with Barker that sexual conditions such as are presented in *Albugo Bliti* are simple enough for the lowest types of Ascomycetes, because the differentiation of ooplasm and periplasm, with the accompanying coenocentrum and the extreme specialization of the antheridium, all characters almost universal in

the Peronosporales, are very much in advance of the sexual organs of Monascus, Dipodascus, and Eremascus.

Barker suggests that the egg of the Peronosporales may be cut out by a film of kinoplasm derived from the spindles of dividing nuclei at the period of oogenesis called zonation. He compares this free cell formation to sporogenesis in the ascus, in an attempt to relate the ascus to the zoosporangium through a gametangium which is homologous with the latter. Stevens and I studied carefully the origin of the egg membrane in Albugo and came to the conclusion that its formation has no connection with spindle fibers. In the first place, zonation is not universal among the Peronosporales; and again, it is largely a matter of chance that the nuclei are in mitosis at that time. Then too, the differentiation of the ooplasm is intimately connected with general dynamic activities of the cell, the same activities that develop the coenocentrum. Zonation is an incident to these more fundamental events. Ooplasm is dense or alveolar, while periplasm becomes very coarsely vacuolate because practically all of the large vacuoles are forced to the periphery by the accumulation of ooplasm around the coenocentrum. The plasma membrane which delimits the egg is formed around the dense ooplasm and becomes bounded by the large vacuoles outside. It is probable that this area of kinoplasm splits along a line of vacuoles between the ooplasm and periplasm and the cell wall is deposited between the two opposed plasma surfaces. These add secondary layers to both sides of the primary wall. The differentiation of the egg then bears no resemblance to the cutting out of ascospores by aster fibers, but on the contrary, recalls and agrees with the activity of vacuoles during the segmentation of the protoplasm in the sporangium of the molds, the oogonium of Saprolegnia, and the zoosporangium.

We are justified in emphasizing these details, since Barker lays stress on the zoosporangium as the possible homologue of the ascus. It should also be noted that the preliminary fusion of nuclei in the ascus is a peculiarity that cannot be lightly passed over in comparisons of this character, where agreement in cytological detail is absolutely necessary to establish homologies.

It is difficult to handle Dipodascus (Juel, 1902) at present because some details of nuclear activities are lacking both at fertilization and during sporogenesis. It seems probable that the development from the fusion cell, which is essentially a zygote, is sporophytic in character and may look forward to the formation of ascogenous hyphae and asci, in which case more information on the methods of spore formation is much to be desired.

The writer sees much in the conditions in Monascus and Dipodascus to support his view that the ancestral sexual organs of the Ascomycetes were coenogametes of the type illustrated by the molds; that the ascocarp is a sporophytic development entirely independent of possible analogous generations in other groups of fungi (*e. g.*, the so-called promycelium of *Phytophthora omnivora*); and that the ascus is a new form of sporangium, connected

with a new generation, and only resembles earlier types (zoosporangium or the sporangium of molds) in such externals as are natural in structures developed terminally.

The evolution of coenogametes may then proceed along three or more divergent lines:

1. There may be the differentiation and utilization of a periplasm to assist in the formation of a thick walled resting spore which with the reduction of the gamete nuclei to one gives the line of ascent in the Peronosporales.

2. The segmentation of protoplasm in the gametangium around several coenocentra to form as many eggs, together with the gradual reduction of the number of gamete nuclei to one for each egg, presents conditions as in the Saprolegniales.

3. In the Ascomycetes superfluous protoplasm may be disposed of through a conjugating tube (Pyronema) or by a sterile cell (Monascus), processes which might lead to the development of trichogynes (lichens and Laboulbeniaceae); and besides these activities there is also the tendency towards nuclear reduction which results in uninucleate gametes (Sphaerotheca).

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EXPLANATION OF PLATES IX AND X.

The material, fixed in weak chrom-acetic acid, was cut 3μ thick and stained on the slide with safranin and gentian violet. All figures were sketched with an Abbé camera under the Zeiss apochromatic objective 2 mm. or 1.5 mm. in combination with compensation oculars. The magnification is as follows: Fig. 1, 250 diameters; Figs. 2–5, 12, and 13, 500 diameters; Figs. 6–9, 1,500 diameters; Figs. 10, 11, 14–29, 1,000 diameters; Figs. 30–35, 667 diameters.

PLATE IX.

(Figs. 1–15 illustrate oogenesis.)

FIG. 1. End of hypha about to form an oogonium.

FIG. 2. Young oogonium, nuclei approaching spirem.

FIG. 3. Central vacuole forming.

FIG. 4. Central vacuole; nuclei in spirem.

FIG. 5. More advanced than Fig. 4; nuclei in mitosis.

FIG. 6. Details of nucleus in spirem condition.

FIG. 7. Metaphase of mitosis; spindle intranuclear; nucleolus outside of spindle; three chromosomes shown.

FIG. 8. Mitosis just after the splitting of chromosomes at nuclear plate; nucleolus outside the spindle.

FIG. 9. Anaphase; two groups of chromosomes, four in each group, at the poles.

FIG. 10. Oogonium after mitosis with twice as many nuclei as previous to that event.

FIG. 11. Oogonium older than in Fig. 10; nuclei degenerating.

FIG. 12. Formation of egg origins under low magnification (500 diameters); coenocentrum in center of each egg origin.

FIG. 13. Egg origins older than in Fig. 12; coenocentra with conspicuous radiations.

FIG. 14. Coenocentrum before the differentiation of the egg origins; radiations plain; nucleus at one side of coenocentrum; other nuclei degenerating in the cytoplasm.

FIG. 15. Similar to Fig. 14; two coenocentra; nucleus at the side of each; many nuclei degenerating in the cytoplasm.

PLATE X.

(Figs. 16–29 illustrate oogenesis.)

FIG. 16. Egg origin just before rounding off to form egg; conspicuous coenocentrum with nucleus at the side.

FIG. 17. Young egg, nucleus larger than in Fig. 16.

FIG. 18. Young egg; coenocentrum without radiations.

FIG. 19. Egg, older than in Figs. 17 and 18; nucleus larger.

FIG. 20. Egg with nucleus extended toward coenocentrum, which has almost disappeared.

FIG. 21. Mature egg; large nucleus; coenocentrum disappeared.

FIG. 22. Young binucleate egg, the two small nuclei close to the smaller coenocentrum.

FIG. 23. Young egg with two coenocentra, each accompanied by a nucleus.

FIG. 24. An exceptionally large binucleate egg with prominent coenocentrum.

FIG. 25. Egg with two nuclei lying over one another, both extended toward the coenocentrum.

FIG. 26. Binucleate egg with the nuclei at a distance from one another.

FIG. 27. Binucleate egg with the nuclei close together.

FIG. 28. Trinucleate egg, the three nuclei lying close together.

FIG. 29. Trinucleate egg, the three nuclei at a distance from one another.

(Figs. 30–35 illustrate sporogenesis.)

FIG. 30. End of sporangium showing development of central vacuole.

FIG. 31. Portion of cross section of sporangium, central vacuole well developed.

FIG. 32. Early stage of segmentation; cleavage furrows running from central vacuole to periphery.

FIG. 33. After cleavage furrows have reached periphery, spore origins forming.

FIG. 34. Spore origins older than in Fig. 33.

FIG. 35. Zoospores in sporangium.

THE MACCHIE OF THE NEAPOLITAN COAST REGION.

J. Y. Bergen.

(WITH FOUR FIGURES)

It has occurred to the writer, after some two years of observation of the xerophilous shrubs and undershrubs of the territory surrounding the bay of Naples and of the islands of Ischia and Capri, that a brief account of this peculiar flora and of its conditions of existence might interest American botanists. Most of the literature of the subject is to be found in Italian botanical periodicals, or in monographs not readily accessible to the American student, and the vegetation in question is well worthy of study, since it constitutes a series of well-defined plant societies of a pronounced, though not extraordinarily xerophytic, character.

The soils of the mainland near Naples generally consist of weathered tufa, of decomposed volcanic scoria and pumice, or of disintegrated lavas of many kinds. Much decomposed vegetable matter is often present. As a rule the soils are deep, warm, and fertile loams, though occasionally feldspathic rocks are found to have been reduced by the action of steam impregnated with sulphur dioxide to a white clay, which is rather sterile. Yellow brick clays sometimes occur.

The soils of Ischia are in general not dissimilar to those of the mainland. Capri is underlaid by a moderately pure limestone, which outcrops in many places, but is usually covered with a somewhat shallow clayey loam, derived in the main from the decomposition of the subjacent limestone.

The climate in the neighborhood of Naples is characterized by mild rainy winters, during which there are few frosts at sea level, and long, hot, and dry summers. The greatest vegetative activity is therefore found in the months from October to May inclusive, and many annual plants complete their growth and blossom at some time during the period from November to April inclusive. Areas which are tenanted mainly by annual

species or by herbaceous perennials, therefore, often present hardly any appearance of plant life during the drier months.

Outside of the many orchards and groups of walnut trees, together with occasional carefully preserved woodlands of small extent, the country is generally treeless. Thickets and considerable tracts covered with undershrubs form, however, an important feature in the physiognomy of the region, as they do of much of the Mediterranean coast, from Spain to Syria. These *macchie*, as they are called in the mainland of Italy and most of the Italian islands immediately about the bay of Naples, usually occupy small tracts of uncultivable land. These are often on steep hillsides, hilltops, or the flanks of mountains, at a height ranging from fifty to more than five hundred meters above sea level.

The plant societies of the macchie consist of many shrubby species, largely Leguminosae and Cistaceae. Most of the commoner species are indigenous, and a few, such as *Cistus salvifolius* L., *Daphne Gnidium* L., and *Euphorbia dendroides* L., are highly characteristic Mediterranean species.

The macchie of the island of Ischia consist of the following species and varieties:[1]

(1) Myrtus communis L.; (2) Pistacia Lentiscus L.; (3) Quercus Ilex L.; (4) Quercus pubescens Willd.; Phillyrea variabilis Timb. & Lor., (5) var. media L. and (6) var. angustifolia L.; (7) Arbutus Unedo L.; (8) Calycotome villosa Link.; (9) Cytisus triflorus L'Her.; (10) Cytisus monspessulanus L.; (11) Coronilla Emerus L.; (12) Spartium junceum L.; (13) Cistus monspeliensis L.; (14) C. salvifolius L.; (15) C. villosus L.; (16) Lonicera implexa Ait.; (17) Rosa sempirvirens L.; (18) Erica arborea L.; (19) Rhamnus Alaternus L.; (20) Daphne Gnidium L.; (21) Smilax aspera L., var. mauritanica Desf.; (22) Clematis Flammula L.

The list for the island of Capri is similar to that above given with the omission of numbers 5, 6, 10, and the addition of the following species:

Pinus halepensis Mill.; Smilax aspera L.; Quercus Cerris L.; Q. Aegilops L.; Thymelaea hirsuta Endl.; Phillyrea variabilis Timb. & Lor., var. latifolia L.; Euphorbia dendroides L.; Euphorbia spinosa L.; Rubus

[1] Slightly modified from the list given by GUSSONE, Enumeratio Plantarum Vascularium in Insula Inarime, p. X. Naples, 1854.

discolor W. & N.; Rosmarinus officinalis L.; Ulex europaeus L.; Calycotome spinosa Link.; Cytisus hirsutus L.; C. spinescens Sieb., var. ramosissimus Ten.; C. Laburnum L.; Colutea arborescens L.; Anthyllis Barba-Jovis L.; Ceratonia Siliqua L.

Several of these species are also found in Ischia, but as Gussone has not included them in his enumeration of the macchie of that island, I have not ventured to put them in.

On the mainland, from Cape Misenum to the end of the Sorrento peninsula, the macchie consist mostly of some combination of the elements found in the preceding lists. A partial idea of the numerical proportions of the species which compose these plant societies may be gathered from enumerations to be given in a subsequent paper on this topic.

A typical Neapolitan macchia does not usually contain half of the species comprised in either of the lists above given, and may even consist of but three or four species, as is the case on the flanks of Monte Nuovo, at the base of Cape Misenum. Here the undershrubs are so scattering as to give no appearance of a thicket, and are in general less than a meter in height. The prevalent species are *Spartium junceum*, *Cistus salvifolius*, *Pistacia Lentiscus*, and *Erica arborea*. In other cases, as in Capri, at the foot of Monte Solaro, near the so-called Baths of Tiberius, from the abundance of such large shrubs as *Arbutus Unedo* and well-grown saplings of *Quercus Ilex*, much of the thicket is not less than three meters in height. It is also, in this instance, peculiarly difficult to traverse, on account of the abundance of such climbers as *Smilax aspera*, *Clematis Flammula*, and the very prickly, herbaceous, trailing *Asparagus acutifolius*.

What Drude calls *Bestände*, and Hult, Kjellman, and others call "plant formations,"[2] rarely occur among the Neapolitan macchie. Arid mountain slopes sometimes show patches of Spartium almost unmixed with other undershrubs, and Cistus of a single species sometimes covers considerable areas, to the exclusion of other woody forms, but during the wet season many herbaceous plants occur intermingled with these species.

A large proportion of the shrubs which constitute macchie

[2] WARMING, Lehrbuch der ökologischen Pflanzengeographie, zweite Auflage. Berlin. 1902, p. 9.

are evergreen, but some are winter deciduous and others are summer deciduous.

Few areas can be found in which thickets are allowed to grow undisturbed. All of the shrubs which have much fuel value are cut to the ground and used for heating ovens and even for firing limekilns or kilns of tile or coarse earthenware.

It should be kept in mind that herbaceous vegetation flourishes vigorously among the macchie, during all but the driest months. A large proportion of the 700 species of phanerogams and vascular cryptogams which occur in Capri, of the 900 or more species of Ischia, and of the 1,000 or more of the mainland immediately surrounding the bay of Naples may be found among the plant societies which constitute the macchie of the region.

In attempting to look up the literature of the subject of this paper the writer has been impressed by the apparent lack of any moderately full bibliography of the topic. Much material of the sort exists, but it does not appear to have been systematically catalogued for the Neapolitan region, as it has been for other Mediterranean territory. A large number of titles will be found in Beck von Mannagetta's "Vegetationsverhältnisse der illyrischen Länder," pp. 25–45. Many are also given in Wilkomm's "Pflanzenverbreitung auf der iberischen Halbinsel," pp. 23–27; but each of these books naturally refers mainly to the works which relate more or less directly to its own region.

The list at the close of this article, for which the author is indebted to his friend, Sig. Giovanni Ettore Mattei, librarian of the Royal Botanic Garden at Naples, contains some of the most important titles of works which will aid in the study of Italian macchie.

ENUMERATION OF PLANTS IN MEASURED AREAS.

In order to give a somewhat definite idea of the completeness with which the ground is covered in macchie of various types and of the relative abundance of different species, the author has counted the individual shrubs occurring in plots of ten meters square. The task of counting was no easy one, as in many cases each bushy clump had to be uprooted in order to ascertain

whether it consisted of more than one individual. Of course single areas of no greater extent than a hundred square meters would in no case contain all the characteristic species of the macchie of a region, but the attempt was made, in every instance, to select fairly representative plots.

The following results were obtained:

A.

Capri, on northwest slope of Monte Solard, about 350 meters above sea level.

Name.	No. of Individuals.
Spartium junceum L.	106
Cistus villosus L. & *C. salvifolius* L.	603
Myrtus communis L.	15
Daphne Gnidium L.	4
Pistacia Lentiscus L.	1
Erica arborea L.	1
Olea europea L.	1
Quercus pubescens Willd.	1

The predominating shrub in this locality was the Spartium, leafless as seen in late summer. It occasionally reached a height of 1.5^{m}, with a spreading top. On the whole the macchia was scattered and open, most of the shrubs not much more than waist-high. The Cistus ranged from 0.2 to 0.5^{m} in height. At irregular intervals of fifty, one hundred, or more meters were scattered individuals of *Pinus halepensis* L. 2 to 4^{m} high, with thin foliage, which casts but little shade.

Although no specimens of any of them occurred in the plot just described, there are several species of undershrubs in the immediate vicinity which assume the pillow or cushion form (*Polsterpflanzen*) so common in some alpine floras. The most noticeable of these are *Cytisus spinescens ramosissimus*, *Euphorbia spinosa*, and *Satureia montana*.

B.

Capri, at foot of Monte Solaro (near stairs to Anacapri), perhaps about 130^{m} above sea level.

Name.	No. of Individuals.
Arbutus Unedo L.	464
Erica arborea L.	12
Quercus Ilex L.	12
Quercus Ægilops L.	12
Clematis Flammula L.	16
Smilax aspera L.	200
Coronilla Emerus L.	812
Myrtus communis L.	124
Cistus villosus L. & *C. salvifolius* L.	24
Pistacia Lentiscus L.	44
Rhamnus Alaternus L.	12

The shrubs in this locality formed a close and rather high thicket. Its denseness was in places at least twice that of the measured area *B*. The *Arbutus* was the predominating species from its large numbers and its size, some of the shrubs being 2.4^{m} high. The *Q. Ilex* in places reached the height of 4^{m}, but in general was somewhat over 2^{m} high. The *Coronilla* and the *Erica* showed themselves to be decidedly shade plants, reaching their greatest dimensions and appearing most vigorous when protected by the shade of taller species, *e. g.*, the *Arbutus*.

The much greater luxuriance of vegetation in *B* than in *A* is not mainly due to the lower altitude of *B*, but rather to the richer soil and better water supply. The soil of *A* was a thin layer of clayey loam, derived mainly from the weathering of the subjacent solid limestone, while that of *B* was of similar chemical and physical character, but deeper, and underlaid by a talus of small fragments of limestone (*fig. 1*).

C.

Pozzuoli, on exterior slope of Solfatara hill, facing S.E., altitude over 120^{m} above sea level.

Name.	No. of Individuals.
Myrtus communis L.	800–1200
Cistus salvifolius L.	190
Calycotome villosa Link	143
Erica arborea L.	42
Spartium junceum L.	33
Inula viscosa Ait.	27
Quercus pubescens Willd.	13
Quercus Ilex L. var.	10
Clematis Flammula L.	1
Arbutus Unedo L.	1

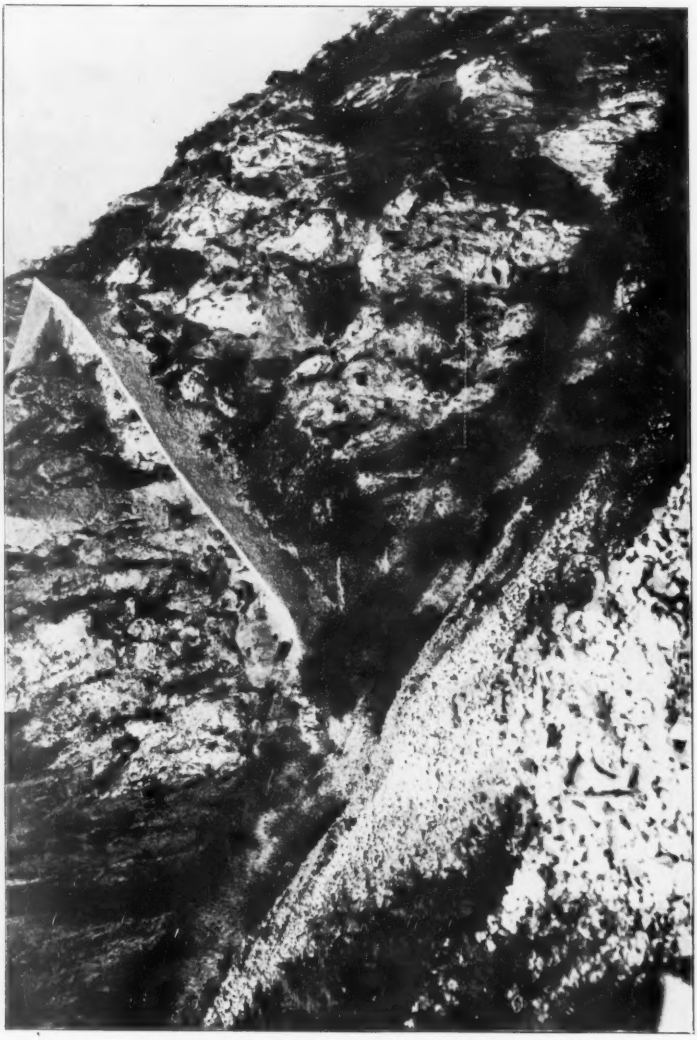

FIG. 1. — Macchia near stairs to Anacapri. Spartium formation in the center. At lower right hand, beginning of Arbutus macchia described under B.

This plot (*fig. 2*) was examined November 12, at which time the Arbutus and Inula were in blossom, the Myrtus and Clematis in fruit, and the other shrubs neither in flower nor in fruit. In numbers the Myrtus greatly exceeded all the other species combined, and indeed was in places so matted that it would have been impossible to count the individuals, without carefully digging up the entire mass and separating it into its factors. The number above given is therefore only an estimate. Most of the specimens were less than 50^{cm} and many only 20–30^{cm} high. The oaks were young growths from the stumps of saplings recently cut to the ground, and were therefore in no case more than 2^{m} high. The Calycotome appeared as a rapidly growing spiny shrub of 2^{m} or less in height, many of the specimens being seedlings of about a year old. The Spartium and the Inula each reached a maximum height of about 1.5^{m}; the latter a straggling undershrub, woody below, but with all the younger portions of the stems still herbaceous.

The Inula is not primarily a characteristic shrub of the macchie, but rather of the cliffside societies, but about Pozznoli it is a frequent companion of the shrubs in the list above given.

The soil of this locality was of volcanic origin, composed of finely divided ejecta from the crater of the Solfatara, with some of the feldspathic rocks decomposed by the action of sulfur dioxid into a white earth which is rather characteristic of the region. The mixture was porous and fairly warm, but by no means fertile.

D.

Monte Gauro, near Pozzuoli, east side of crater, west slope. Somewhat less than 200^{m} above sea-level.

Colutea arborescens L.	101
Spartium junceum L.	22

E.

Monte Gauro, east side of crater, east (outside) slope. About 200^{m} above sea level.

Quercus pubescens Willd.	32
Spartium junceum L.	22
Crataegus Oxyacantha monogyna Jacq.	11
Rubus discolor W. & N.	8
Colutea arborescens L.	3
Clematis Flammula L.	3
Cistus salvifolius L.	62

FIG. 2.— Macchia described under C, from exterior slope of Solfatara near Pozzuoli. Most of the broad leaved shrubs are Arbutus or Inula. The narrow leaved ones are Erica.

The soil in both *D* and *E* was a rich loam, formed of decomposed tufa, deeper in the former locality than in the latter. The plant-society of *D* is a rather exceptional one, as the Colutea (here sometimes 2.5^{m} high) is a rare or local shrub. Large

FIG. 3.— Interior of Solfatara near Pozzuoli, with macchia of Myrtus, Erica and Calycotome on north wall of crater.

areas on Monte Gauro are, however, occupied by it, together with a few other species. Near the locality *D* but in scantier soil and more fully exposed to the sun were formations almost wholly composed respectively of *Pistacia Lentiscus* and of *Calycotome villosa*. At a lower level, in rich deep soil, was a consid-

erable tract covered with *Castanea sativa* Mill. (young saplings), and a shrub that appeared to be *Cytisus scoparius* Link.

The society *E* presented the aspect of a very open thicket, with the oaks predominating over all the other species, as many of them were about 3.5^{m} high. The Crataegus is not a common ingredient of the macchie about Pozzuoli, but frequently is encountered singly on the edges of banks, overhanging roads or cliffs. *Cistus salvifolius* when found growing among or under trees or tall shrubs, as in the societies *B* and *E* does not reach anything like its normal development, as, according to Beck (*op. cit.*, p. 126), it cannot bloom without receiving at least 0.23 of the total light intensity of the regions where it grows.

The areas *D* and *E* were measured and counted on December 20, and it is an interesting illustration of the indefiniteness of the seasons, and the imperfectly tropophytic character of the vegetation about Naples that the Colutea in many cases bore at the same time dry ripened pods, new leaves, old leaves, and a few flowers, although the regular flowering season is May and June. So too the Crataegus, which regularly blossoms in April and May, showed on December 20, in a few instances, new leaves, old leaves, blossoms, and ripe fruit on the same branches, This in spite of the fact that the winter of 1902 has been a cold one for the region, though without frost up to the date above mentioned.

In summing up the enumerations, it may be said that *B* and *C* are the most typical Neapolitan macchie, but the others are good examples of the way in which the grouping of species varies with soil, exposure, and other circumstances.

Side by side with the flora of the Neapolitan macchie there grows another (*fig. 4*), consisting, like the former, largely of woody plants, but for the most part distinct from it. I refer to the larger plants that partially clothe most of the cliff sides and many of the older tufa walls. The principal species are:

1. *Artemisia arborescens* L.
2. *Artemisia variabilis* Ten.
3. *Helichrysum rupestre* DC.
4. *Helichrysum litoreum* Guss.
5. *Achillea ligustica* All.
6. *Inula viscosa* Ait.
7. *Centranthus ruber* Dec.
8. *Spartium junceum* L.
9. *Medicago arborea* L.
10. *Opuntia Ficus-indica* Mill.
11. *Matthiola rupestris* Dec.
12. *M. incana* Dec.
13. *Satureia graeca* L.
14. *Euphorbia dendroides* L.

FIG. 4.— Flora of a nearly vertical cliffside at Pozzuoli. Suppose the cut to be divided into four equal vertical bands, numbered from left to right.

1. Top, *Olea sativa;* next, *Spartium junceum;* next, *Artemisia arborescens;* bottom, *Artemisia variabilis.*

2. Two-fifths distance from top to bottom, *Opuntia Ficus-indica* and *Matthiola incana.*

3. Top, *Pistacia Lentiscus* and *Asparagus acutifolius;* next, *Artemisia variabilis.*

4. Top, *Opuntia Ficus-indica;* next, *Artemisia variabilis;* next, *A. arborescens;* bottom. *Opuntia Ficus-indica.*

Of these plants only the Inula, the Spartium, and the Euphorbia are of common occurrence in the macchie. Generally speaking, all the species mentioned are much more xerophytic than their neighbors on more level ground. The Compositae (nos. 1–6) are all aromatic with essential oil; nos. 1, 3, and 4 are densely sericeous or tomentose, and nos. 3 and 4 have strongly revolute leaves, the margins almost meeting; no. 2 has fleshy, linear leaf segments, and no. 6 has pubescent varnished leaves. Of the remaining species, the Spartium and the Euphorbia are discussed in a later paragraph, the Opuntia (an introduced plant) is one of the most succulent of the genus, the Medicago has small pubescent leaves, and the Matthiolas are hoary pubescent.

(*To be concluded.*)

BRIEFER ARTICLES.

NUTATION IN BIDENS AND OTHER GENERA.

(WITH FOUR FIGURES)

THERE has long been a belief, regarded by many as a mere superstition, that the sunflower turns with the sun. In 1898[1] and in 1900[2] Schaffner published a series of illustrations and observations demonstrating conclusively that such a nutation exists. He makes no mention of any plant other than species of Helianthus that exhibit this

FIG. 1.— A single plant of *Bidens frondosa*, taken at 10 A. M., showing the eastward morning nutation.

[1] Observations on the nutation *Helianthus annuus*. BOT. GAZ. **25**: 395-403. 1898.

[2] The nutation of Helianthus. BOT. GAZ. **29**: 197-200. 1900.

nutation, nor am I aware that any other plant has been observed to share with Helianthus the distinction of being a "sunflower."

During the past four or five years my attention has been repeatedly attracted to other genera that exhibited a diurnal motion almost identical in kind with that of Helianthus, and almost if not quite as marked. The accompanying illustrations of *Bidens frondosa* show the extent of

FIG. 2.— Group of *Bidens frondosa*, taken in the morning, to show prevailing eastward nutation (to the left).

the nutation in this species. *Fig. 1* shows one plant in the morning at 10 o'clock. The exposure occupied about one minute and it is evident that the curvature is not due to the wind, nor had the wind been blowing. I have repeatedly observed this same plant in the afternoon and found the westward nutation fully as marked as is the eastward in this illustration. *Fig. 2* shows a mass of plants of the same species in the morning, and illustrates the extent to which this habit preponderates among individuals. The same group taken at four o'clock the same afternoon is shown in *fig. 3*. This is simply a small portion of a roadside patch of weeds that extended for miles. Several counts showed that

between 95 and 98 per cent. of the individuals partook of this motion, some to a considerably more marked extent than others.

Agreeing with the observation of Schaffner for Helianthus, the nutation is much stronger when the ground is moist and the air warm and dry. *Fig. 4* is taken with the camera facing away from the sun, as is shown by the shadow of the camera itself near the lower left hand

FIG. 3.— The same group shown in *fig. 2*, taken in the afternoon, to show strong westward nutation (to the right).

corner of the photograph. The almost universal sunward (westward) nutation here is strikingly shown. A photograph taken with the camera facing westward would have had the same general aspect if taken in the forenoon.

The nutation in *Ambrosia artemisiaefolia* is fully as marked as in Bidens, as I have determined by a large number of marked plants kept under observation during night and day. Some of these I have watched for many days, as they lay in my path to and from the class-room. Here the maximum eastward nutation is at about 9 A. M. Noon finds

the stem erect, while at 7 or 8 P. M. the maximum westward nutation occurs. The plant begins to assume the erect position soon after sunset, and is usually quite erect by 10 or 11 P. M., and remains so until approaching sunrise.

Outside of the Compositae I find a nutation, though less marked, in Amaranthus, particularly in the younger plants, and in many of the

FIG. 4.—*Bidens frondosa*, taken with camera facing east. All plants facing the camera and the sun.

Leguminosae. Doubtless it is to be found in many other families. Among the Leguminosae, Lespedeza, Melilotus, Medicago, and Trifolium are striking in nutation. *Melilotus alba*, for example, is particularly interesting, as the marked nutation strikingly affects the landscape as seen in going and returning from a day's drive.

It is my purpose here simply to call attention to the diurnal bending or nodding of the stem. In addition to this there are of course very complicated changes in leaf position.—FRANK LINCOLN STEVENS, *Agricultural and Mechanical College, Raleigh, N. C.*

CURRENT LITERATURE.

BOOK REVIEWS.

Forestry and wood.

ALTHOUGH primarily a volume on forestry from the standpoint of political economy, the work by Fernow entitled the *Economics of forestry*[1] contains much that is of botanical interest. Under the heading, the forest as a condition, there is an excellent discussion on the influence of the forest upon the climatic conditions within its own limits and beyond. Emphasis is laid upon the fact that this influence is in no way comparable to the influence of the ocean, great air-currents, and extensive mountain ranges, but rather that the forest can modify only locally the effects of this general climate. The forest cover has a tendency to reduce the extremes of high and low temperature, but how far this difference is felt outside the forest has not yet been determined. The author holds that there is not, as yet, sufficiently reliable rainfall measurements obtainable to settle the question whether or not the forest influences the amount of rainfall. There are more reliable data, however, which show that as a wind-break the forest does modify the climate locally.

Other topics treated under the same general heading are the influence of forests upon the distribution and character of the water-flow, upon the mechanical conditions and erosion of the soil under cover, upon the health conditions, and upon the ethics of a people. In the chapter on the definition of forest and forestry one finds that forest knowledge is divided into three headings, viz.: the economic aspects (the condition), the technical aspects (the crop), and the business aspects (the revenue). The headings are ultimately divided into twenty-seven different branches, all of which are necessary to the comprehensive understanding of the subject.

In comparing forestry with agriculture the author says that in agriculture the factor of labor is most important, nature second, and capital last; in the forestry business the reliance on nature is greatest, on capital next, while labor plays a less important part. Nature unassisted has produced the virgin forest, but agricultural production is almost entirely dependent on human effort. To emphasize this difference it is shown that in Germany fifteen to fifty laborers are continuously employed on 250-acre farms as against one to three on the same acreage of forest.

[1] FERNOW, B. E., Economics of forestry, a reference book for students of political economy and professional and lay students of forestry. 8vo. pp. xii + 520. New York: Thomas Y. Crowell & Co. 1902.

The chapter on the natural history of the forest contains an interesting discussion of the physical and biological relations of trees and forests. Other general topics treated are sylviculture, forest economy, forest policy, forest policies of foreign nations, forest conditions, and the forestry movement in the United States. The book is not intended as a popular discussion, but to supply a lack in the professional literature of economics in the English language. It is interestingly written and embodies so much general knowledge that it deserves to have a wide circulation.

UNDER THE general title *Wood*, Boulger[2] has collected in one volume many facts concerning both the scientific and commercial aspects of the subject. In the first chapter the origin, structure, and development of wood and the uses of wood are discussed. Then follows a comprehensive and valuable key for the determination of woods. Of more than usual interest is the treatment of the defects of woods, the selection, seasoning, storage, and durability of woods, the application of special woods, and supplies of wood. In part two is found an alphabetical list of woods, with facts concerning their sources, character, and uses.—H. N. WHITFORD.

MINOR NOTICES.

POSTELSIA[3] is a collection of seven botanical essays, four of which are phycological in character. The first is the *Uses of marine algae in Japan*, by K. YENDO. There are probably no people that make such extensive use of seaweeds as the Japanese. We are given a list of perhaps twenty-five forms that have a place in the life, principally of the peasantry, some as staple foods and others as delicacies, condiments, and decorative plants. There is even an extensive export trade, chiefly with China, for laminaria and agar-agar, which amounts to more than 30,000,000 lbs. a year. With each form is a brief account of its uses and the paper is illustrated with three Japanese prints. *Algae collecting in the Hawaiian Islands*, by JOSEPHINE E. TILDEN, is a very readable account of a summer spent among these islands, whose marine flora, rich in green and red algae, presents the sharpest sort of contrast to the luxuriant brown algae of the Pacific coast. Yendo has a second paper entitled *The distribution of marine algae in Japan*. He describes the varied ocean currents that bathe the Japanese archipelago and give to it such great extremes of temperature that a sub-arctic marine flora may be found almost side by side with a tropical. Japan with its bold shores and many bays and indentations furnishes wonderful variations in conditions, and will prove a magnificent field for a study of the factors that determine the distribution of algae. There

[2] BOULGER, G. S., Wood, a manual of the natural history and industrial application of the timber of commerce. 8vo. pp. viii + 369, *figs. 66. pls. 4*. London: Edward Arnold. 1902. $3.00.

[3] POSTELSIA. The Year Book of the Minnesota Seaside Station. 1901. 8vo. pp. 220. *pls. 26*. St. Paul: The Pioneer Press. 1902.

is also a brief account of the marine laboratory at Misaki. The last essay is by Professor MacMillan on *The kelps of Juan de Fuca*. There are seventeen genera of kelps found in the Straits of Fuca, which is more than half of the total number in the Laminariales. The habits and many of the structural peculiarities of these are described in a popular style and illustrated with several excellent figures.— B. M. DAVIS.

PROFESSOR MÖBIUS[4] has published in book form his laboratory dire tions for beginners. The book contains eighteen exercises, each intende to occupy about three hours. Directions are given for making sixty-five preparations, of which forty-two are phanerogams and twenty-three cryptogams. Almost without exception, the methods are those employed for examining fresh material, no microtome being used and almost no attention being given to imbedding or staining. In this country subject-matter like that contained in this book is usually presented by the teacher in the laboratory CHARLES J. CHAMBERLAIN.

NOTES FOR STUDENTS.

MONTEVERDE[5] states, in a preliminary notice, that protochlorophyll is not a yellow pigment, but is like chlorophyll in color and fluorescence. Etiolated leaves contain a certain amount of this substance, which begins to go over into chlorophyll as soon as these are brought into the light. In light, protochlorophyll is formed as fast as it changes into chlorophyll, and if green plants are placed in darkness this process sometimes continues until the green of the leaves is heightened by the accumulation of the former compound.— BURTON E. LIVINGSTON.

MOLISCH[6] finds the phosphorescent bacterium, *Micrococcus phosphoreus* Cohn, to be common and widely distributed instead of rare and sporadic as generally supposed. It is found upon meats in refrigerators and cold-storage houses, abattoirs, butcher shops, and kitchens into which flesh of slaughtered animals is regularly brought. To secure this species for study it suffices to dip a piece of fresh beef, veal, or pork into a 3 per cent. NaCl solution, to lay it in a dish half immersed in the same solution and keep it at 9–12° C. in a moist chamber. 89 per cent. of beef tested in this way showed luminescence. As the species dies quickly at the temperature of the human body it probably cannot be injurious if eaten. A revised description is given with illustrations.— C. R. B.

[4] MÖBIUS, M., Botanisch-mikroskopisches Praktikum für Anfänger. 8vo. pp. ix + 2. *figs.* 12. Berlin: Gebrüder Borntraeger. 1903.

[5] MONTEVERDE, N. A., Das Protochlorophyll und Chlorophyll. Bull. Jard. Imp. Bot. St. Petersburg **2**: 181–182. 1902.

[6] MOLISCH, HANS, Ueber das Leuchten des Fleisches, insbesondere todten Schlachtthiere. Bot. Zeit. **61**[1]: 1–18. *figs.* 5. 1903.

THE PRINCIPAL points in our knowledge of the dendritic monocotyledons, together with some new observations, form the subject of a recent paper by Schoute.[7] On account of the slow progress of primary growth in thickness at the vegetative point, the stem is usually shaped like an inverted cone at the base, as in Pandanus; here prop-roots serve to support the stem. In the Liliiflorae, however, the inverted cone of primary growth is surrounded by a zone of secondary growth, giving the stem a cylindrical form at the base. Measurements of stems of Dracaena and other genera are given, which establish this point. Branches appear when apical growth is stopped by production of a terminal flower cluster; the growth of the branches repeats the process observed in the main stem.—M. A. CHRYSLER.

SCHUMANN has made an extensive comparative study of the ovulate flowers of the Coniferales (including Ginkgo).[8] Little attention is given to the earlier developmental stages, but the later stages and the mature flowers are described in great detail, and many suggestive comparisons are made. Teratology is not regarded as of much value in elucidating morphological relations or establishing homologies. Schumann finds that the Cupresseae are related to the Taxodieae, Sequoia being of living forms nearest the point of contact. This does not mean that Sequoia is the starting-point for the Cupressineae, but that the ancestral form must have been similar and related to Sequoia. This supports Potonié's theory that the Taxodieae appeared before the Cupresseae.—CHARLES J. CHAMBERLAIN.

IN A STUDY of the changes taking place in glucosides during metabolism, Weevers[9] finds that salicin is removed from the leaves of Salix to the bark in the dark and increases again in the leaves in light. Saligenol appears only in small quantities where salicin is decreasing in amount, quantities not large enough to account for nearly all of the salicin removed. There is found, however, an abundance of catechol in tissues from which the glucoside has disappeared, and in amounts sufficient to justify the supposition that salicin breaks down into glucose and catechol, saligenol being only an intermediate product. An enzyme active in this process could not be extracted, but it was found necessary to kill the tissues immediately in order to prevent a decrease in salicin content. Thus, the living protoplasm or a substance destroyed by boiling seems to be necessary. When glucose and catechol are produced, the carbohydrate diffuses away and the catechol remains and combines with glucose again when it becomes plentiful. Investigation of the changes in aesculin in germinating horse-chestnuts and of gaultherin in Gaultheria and Fagus were also made, but they are not yet as complete as the others.—BURTON E. LIVINGSTON.

[7] SCHOUTE, J. C., Die Stammesbildung der Monocotylen. Flora **92**: 32–48. 1903.

[8] SCHUMANN, K., Ueber die weiblichen Blüthen der Coniferen. Verhandl. Bot. Vereins Prov. Brandenburg **44**: 5–80. 1902.

[9] WEEVERS, TH., Investigation of glucosides in connection with the internal mutation of plants. Kon. Akad. Wet. Amsterdam **1902**: 295–303. (Nov. 20.)

THE DEVELOPMENT of the spores in Selaginella has been investigated by Denke.[10] As is well known, Selaginella is a refractory object for cytological work. Denke recommends for fixing a mixture of one-third acetic acid and two-thirds absolute alcohol, and Haidenhain's iron-alum haematoxylin, with or without Congo red, for staining. He finds that the cauline sporangia originate from epidermal cells and underlying tissue. In the microsporangium a considerable number of spore mother cells fail to undergo the tetrad division. Such cells lose protoplasmic contents and function like tapetal cells. Microsporangia and megasporangia develop alike up to the spore mother cell stage and in phylogeny probably represent a common structure. In both microspore and megaspore mother cells the spindle is extranuclear in origin, and it appears while the nucleus is still in the resting stage. The spindle soon becomes bipolar and in its behavior recalls the spindle in the spermatogonia of the salamander. The formation of the membranes of the four young spores is peculiar, and, in spite of the attention which has been given to the subject, a conclusive account has not yet appeared. From the cytoplasm of the young spore, two membranes are formed one after the other. At first they grow in contact with each other, but the outer membrane by a more rapid growth becomes separated from the other, and a space appears between them. The inner layer — the mesospore — does not arise by a mere splitting of the outer layer, or exospore, as some have supposed. The delicate wall formed around each of the four young spores at the division of the spore mother cell is dissolved.—CHARLES J. CHAMBERLAIN.

GUIGNARD[11] has obtained some very interesting results from a study of *Hypecoum procumbens*, one of the Papaveraceae. Incidentally triple fusion was found to occur in the embryo sac, and the antipodals show that increase in size and prolonged activity characteristic of the antipodals of Ranunculaceae; but the chief interest centers in the unusual formation of the proembryo. The first division of the fertilized egg is transverse, as is usual, but by the enlargement of the basal cell the apical cell is thrust somewhat to one side and in this position divides at right angles to the first division. At first the two daughter cells of the second division are equal in size, but soon the upper one begins to enlarge, and becomes pyriform and approximately parallel with the origin basal cell. The three-celled proembryo thus consists of two large and inflated and presently vacuolate basal cells, lying side by side, and a small embryonal cell resting between them at their free ends. The two basal cells thus form a suspensor of most unusual origin, and remain very prominent and active during the slow development of the embryo, which is somewhat peculiar, in that it is not initiated by the longitudinal division of the embryonal cell, but by transverse divisions the embryo becomes

[10] DENKE, P., Sporenentwickelung bei Selaginella. Beihefte zum Bot. Centralbl. **12**: 182–199. *pls. 5*. 1902.

[11] GUIGNARD, L., La formation et le développement de l'embryon chez l'Hypecoum. Jour. Botanique **17**: 33–44. *figs. 21*. 1903.

a filament of four or five cells, whose apical and basal cells are the last to divide longitudinally.

The investigation traverses the account of Hypecoum given by Hegelmaier in 1878, and explains some of his inexplicable results, but at the same time introduces a proembryo and suspensor without precedent among angiosperms. The behavior of the suspensor cells after their formation resembles that found by Guignard among the Leguminosae.— J. M. C.

A RECENT PAPER read by H. Marshall Ward[12] before the Royal Society gives the results of a detailed study of the histological features of the germination, infection, and growth of the mycelium of *Uredo dispersa* in the tissue of grasses. The research has been prolonged and has involved thousands of preparations. The paper deals with the behavior of the nuclei, vacuoles, septa, branches, haustoria, and other details of the hyphae up to the commencement of spore formation.

The relations of the hyphae to the cell contents of the host are critically examined and the cumulative evidence not only fails to support Eriksson's "mycoplasm" hypothesis, but is completely subversive of it so far as histological facts are concerned. Eriksson's hypothesis, which refers the epidemic outbreaks of rust to the sudden transformation into the mycelial form of a supposed infective substance, previously latent and invisible in the cytoplasm of the host, is shown to be untenable because the *corpuscules speciaux* of this author are proved to be the cut-off haustoria of the fungus. Eriksson supposes that these *corpuscules* (haustoria) are formed by the hitherto latent germs in the host-cells, growing up in the cells into vesicles, which then pierce the cell-walls, and give rise to hyphae in the intercellular spaces. The present paper shows that Eriksson has entirely reversed the true order of events. The haustoria have been formed by the hyphae, and figures are given showing every stage in their development. The first haustorium may be formed by the infecting tube immediately after its penetration through the stoma, and figures are given showing the remains of the germ-tube outside a stoma, the swelling of its tip over the stoma into an appressorium, the passage through the stomatal cavity, and its development into a vesicular swelling whence the true infection tube arises, which latter may at once put forth a haustorium. In some cases all these latter phenomena are visible in one and the same preparation.— J. M. C.

NATHANSOHN[13] has recently made what seems to be by far the most important contribution to our knowledge of absorption and excretion which has yet appeared. Working with *Codium tomentosum*, he finds that when the plant is transferred from sea-water to a solution of $NaNO_3$, absorption of this

[12] WARD, H. MARSHALL, On the histology of *Uredo dispersa* Erikss., and the "mycoplasm" hypothesis. Read March 12, 1903.

[13] NATHANSOHN, ALEXANDER, Ueber Regulationserscheinungen im Stoffaustausch Jahrb. Wiss. Bot. **38**: 241–290. 1902.

salt occurs up to a certain limit, and then ceases. The limit of absorption seems to be determined by the concentration of the external solution, more solute being absorbed the more concentrated the latter is; but the process never continues until equality of concentration within and without the cells is even approached. This rule does not hold for sea-water, however, in which the alga accumulates $NaNO_3$ to a concentration of 0.02 per cent., although this medium contains only minute traces of the salt.

Further than this, if the alga be placed for a day in a 5 per cent. solution of $NaNO_3$, and then be transferred to a 3 per cent. solution of the same salt, absorption occurs in the stronger solution until the limit for that solution is reached, but after the transfer $NaNO_3$ is lost by the cells until the internal concentration has fallen to the limit for the weaker solution. Since the limit of absorption, even for the stronger solution, lies far below 3 per cent., this outward passage of the salt takes place from a lower to a higher concentration, and therefore in the direction opposite to that in which it should move according to the principles of diffusion of solutes.

The cells contain a large amount of NaCl when taken from sea-water. This salt diffuses outward in $NaNO_3$ solutions until a certain minimum of internal concentration of NaCl is reached, and this minimum limit is lower, the weaker is the external solution of the other salt. The internal concentration of the chlorid never even approaches its external concentration; thus this stoppage of outward diffusion of NaCl is parallel with the cessation of absorption in the case of the nitrate. There is no evidence of accumulation of either salt in the protoplasm, neither is the solubility of either salt different in the cell sap from its solubility in sea-water.

Further, if Codium plants be placed in a solution containing both $NaNO_3$ and NaCl (the latter salt being always of the same concentration, while the former is varied in amount), diffusion of the chlorid takes place either outward or inward according to the concentration of the nitrate without. Thus, if the concentration of $NaNO_3$ is 1 per cent. the plant loses chlorid; if it is 2 or 3 per cent., chlorid is absorbed, and this diffusion occurs whether the chlorid is moving with or against its diffusion tension. The usual principles of diffusion seem not to apply here at all.

This is the first unquestionable evidence that the living membrane in plants can overcome the osmotic pressure of a solute and cause its movement from lower to higher concentration. The phenomenon has been known in animals, however. The paper contains a large amount of suggestive theoretical discussion.—BURTON E. LIVINGSTON.

NEWS.

PROFESSOR DR. E. HEINRICHER, of the University of Innsbruck, will depart for Buitenzorg, Java, in the autumn.

DR. OSCAR EMMERLING has been promoted to an associate professorship of botany in the University of Berlin.—*Science.*

THE TITLE of professor has been conferred upon Drs. Gustav Lindau, Paul Hennings, and Carl Holtermann, of Berlin.

PROFESSOR W. A. SETCHELL, of the University of California, will spend next year in Europe in botanical investigation and travel.

PROFESSOR VOLNEY M. SPALDING, of the University of Michigan, has been given a year's leave of absence, which he expects to devote to travel abroad.

DR. MICHAEL WORONIN, member of the Academy and professor of botany in the University of St. Petersburg, died recently at the age of seventy-five years.

PROFESSOR JOHN M. COULTER, head of the Department of Botany in the University of Chicago, will travel in Europe during the summer and autumn. He sails with his family on May 23.

DR. A. GINZBERGER has been promoted to the position of adjunct in the Botanical Museum and Garden of the Imperial University of Vienna. DR. O. PORSCH has been appointed assistant in the same institution.

MR. A. D. SELBY, botanist to the Agricultural Experiment Station at Wooster, Ohio, who has been in residence at the New York Botanical Garden since December, has been granted a research scholarship of the Garden.—*Science.*

PROFESSOR R. E. SMITH, assistant in botany at the Massachusetts Agricultural College and Experiment Station, has accepted the position of pathologist and assistant professor in botany in the University of California. —*Science.*

FILIBERT ROTH, formerly assistant professor of forestry at Cornell University, and later chief of Forest Reservations in the Department of the Interior, has been appointed professor of forestry in the University of Michigan.—*Science.*

IN THE NEW appropriation to the U. S. Department of Agriculture, $674,930 have been granted to the Bureau of Plant Industry, an increase of

$62,200, and to the Bureau of Forestry $350,000, an increase of $58,140 over last year.

MR. CHARLES A. DAVIS, instructor in forestry in the University of Michigan, has been engaged to prepare a map showing the distribution of forest trees and soil relations for the Ann Arbor sheet of the topographic atlas soon to be published by the United States Geological Survey.—*Science.*

OTTO JAAP, of Hamburg (Mittelstr. 67) has undertaken the publication of *Fungi Selecti Exsiccati*, consisting of new or rare species in series of twenty-five numbers, with printed labels. Heteroecious species are to be represented in their different forms under the same number. The first series consists of forty-two pockets, representing twenty-five species.

THE RANKS of mycologists have suffered an irreparable loss in the death of Dr. A. N. Berlese, which occurred on January 26 at Milan, where he was professor of plant pathology in the Agricultural College. Although only thirty-eight years of age, his name has become well known to the scientific world on account of his extensive work in mycology and plant pathology.

MR. THOMAS HOWELL, editor of the *Flora of the Northwest* and a veteran collector in that region, has been appointed for the coming year field collector and curator of the herbarium for the University of Oregon. He has donated his herbarium to the University. It contains specimens from Oregon, Washington, Idaho, and Alaska to the number of ten thousand or more, many of them being types.

THE MICHIGAN ACADEMY OF SCIENCE, at its recent meeting held in Ann Arbor, elected among its officers for the ensuing year the following botanists: as president, Dr. Frederick C. Newcombe, University of Michigan; as secretary, Dr. James B. Pollock, University of Michigan; as vice-president of the section of botany, Professor B. O. Longyear, Michigan Agricultural College; as vice-president of the section of agriculture, Professor W. J. Beal, Michigan Agricultural College.

M. ÉMILE BESCHERELLE, honorary chief of division of Ministry of Public Works in France, died on February 26 in the seventy-sixth year of his age. For a long time he has devoted his leisure to the study of mosses, in whose taxonomy he had become an acknowledged authority. He had been for some years in feeble health, but continued work to the last upon a *Sylloge*, in which he intended to include all the diagnoses of new species published by him from 1862 to 1902. He had been president of the Botanical Society of France and was corresponding member of the Muséum d'Histoire naturelle, to which establishment he has given his type specimens.

PROFESSOR H. L. BOLLEY, botanist of the North Dakota Agricultural College and Experiment Station, has been appointed special agent for the investigation of the flax crop and flax diseases in Europe. Mr. Bolley will sail the first of June, spend some time in the Netherlands, and then proceed

to eastern Russia, where an extensive study will be made upon the Russian crop, with a view to procuring types of seed which will be valuable for experimental tests in this country. As Agricultural Explorer he hopes to visit all the chief flax-producing regions and to learn much which may be of use to growers in this country. He has already contributed to the promotion of this industry by his investigations. Leave of absence has been allowed by the College, to which he will return.

DR. N. L. BRITTON, director-in-chief of the New York Botanical Garden, and Mrs. Britton returned from Cuba on April 4. During the brief visit made to the island a large collection of herbarium specimens was made, principally from the region around Matanzas, and some desirable specimens were brought back for the conservatories. Valuable assistance was rendered by Mr. John Shafer, curator of the herbarium of the Carnegie Museum, Pittsburg. In addition to the great amount of material secured which will be of great value in the continuation of investigations upon the flora of the West Indies, Dr. Britton was so fortunate as to secure a number of rare botanical books not previously in the library of the garden. Mr. Percy Wilson, museum aid, returned from Honduras March 18, bringing a large collection of living and prepared specimens of plants from the region near Puerto Sierra and Puerto Cortez.—*Science*.

THE DESERT BOTANICAL LABORATORY of the Carnegie Institution will be located at Tucson. Mr. Frederick V. Coville and Dr. D. T. MacDougal, the advisory board of the laboratory, after an examination of the deserts of Texas, New Mexico, Arizona, California, Chihuahua, and Sonora, reported in favor of locating the laboratory near Tucson, and the executive committee of the Carnegie Institution has approved the selection. The building is to be located on the shoulder of a mountain two miles west of the city. This mountain and the adjoining mesas bear an abundant representation of characteristic desert plants. The officers of the University of Arizona and of the Arizona Agricultural Experiment Station have taken a lively interest in the project. The Tucson Chamber of Commerce expressed its appreciation of the importance of the enterprise by donating the site and installing water supply and electric connections, beside rendering other valuable assistance. Plans for the building have been approved, and construction will be begun as soon as the site is prepared. It is expected that the laboratory will be ready for operation about September 1, at which time Dr. W. A. Cannon, the resident investigator, will take up his duties.